Sustainable Requirements for Unconventional Proteins

D1.1

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List of abbreviations

AFSSA	French Agency for Food Safety
ANR	French National Research Agency
AR	Average Requirement
As	Arsenic
ASE	Accelerated Solvent Extraction
BCAA	Branch Chain Amino Acid
BHT	Butylated Hydroxytoluene
CAGR	Compound Annual Growth Rate
Cd	Cadmium
CEN	European Committee for Standardization
Cfu	Colony forming unit
Cu	Copper
DES	Deep Eutectic Solvents
DGE/D-A-CH	German Nutritional Society
EC	European Commission
EFSA	European Food Safety Authority
EoL	End of Life
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization
FBO	Food Business Operator
GDPR	General Data Protection Regulation
GHG	Greenhouse gas emissions
GMP	Good Manufacturing Practices
GRAS	Generally regarded as safe
Ha	Hectare
HACCP	Hazard Analysis & Critical Control Points
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor

Hg	Mercury
HPLC	High-Performance Liquid Chromatography
IL	Ionic Liquids
ISO	International Standards Organization
KD	Distribution Constant for Solvent Extraction Techniques
LCA	Life Cycle Assessment
LCC	Life Cycle Costing
LLD	Liquid-Liquid Extraction
ML	Maximum Level
NADE	Natural Deep Eutectic Solvents
NWR	Nordic Nutritional Recommendation
Pb	Lead
PEF	Pulse Electric Field
PLA	Polylactic acid
PLE	Pressurised Liquid Extraction
PTWI	Provisional Tolerable Weekly Intake
RI	Recommended Intake
SAFA	Sustainability Assessment of Food and Agriculture Systems
SCP	Single Cell Protein
SDG	Sustainable Development Goals
S-LCA	Social Life Cycle Assessment
SSF	Solid State Fermentation
TAMC	Total Aerobic Microbial Count
TM	Tenebrio Molitor
TPP	Three-phase Partitioning
UN	United Nations
UNU	United Nations University
VOC	Volatile Organic Compound
WHO	World Health Organization

Abstract

As the global population continues to rise our current natural resources are coming under increased pressure. Europe's reliance on imported protein sources is likely to become problematic if the population reaches predicted levels by 2050. Europe now runs the risk of a protein gap and there is a pressing need to find new sustainable protein sources that are high quality, healthy and have the desired functional properties to meet end user needs and expectations. The InnoProtein project recognises the urgency of this situation and aims to guide Europe towards protein self-sufficiency. This project seeks to investigate the suitability of unconventional, sustainable protein sources such as single cell proteins (SCPs) including bacteria, microalgae and fungi, as well as entomological alternatives for use as high-quality proteins for food and feed applications. While proteins outside of this spec will be utilised for non-food biobased applications. Additionally, the InnoProtein project has adopted a circular, zero waste approach to protein production and intends to valorise any residual biomass that is produced. An in-depth review of the current state-of the art relating to the sustainable requirements for the InnoProtein project was conducted which examined the social, market, environmental and regulatory aspects of the project. The social requirements of the project were examined and highlighted the health and nutritional needs of the general population at various life stages, as well as, discussing these requirements with regards to pigs, poultry, and trout. Additionally, the social requirements highlight the need of InnoProtein proteins to comply with the toxicological safety limits and states that they must meet specific sensory, physical, and nutritional requirements to ensure their suitability for use in different food and feed applications. With regards to the market requirements this review has found that the insect protein market currently holds the greatest market value, while it is anticipated that there will be significant growth in the microalgae market. The review has also highlighted that the high cost of production is the InnoProtein projects greatest barrier to market entry. Our current protein production practices are now beginning to negatively impact the environment as a result of intensive agriculture and unsustainable food production methods. The utilisation of SCPs and insects as alternative protein sources have a number of sustainable and environmentally friendly benefits. However, protein recovery from these sources can be challenging and traditional extraction methods are no longer sustainable. A move to greener extraction techniques will enable a more efficient and environmentally friendly alternative to protein recovery from these sources. An extensive inventory of regulations, standards, and guidelines for the InnoProtein project has been developed from national, European, and international databases. The purpose of which is to provide guidance for the InnoProtein partners and enabling them to conduct their tasks in compliance with the legislation.

Introduction

With the global population estimated to reach almost ten billion by 2050 (UN, 2017) it is expected that there will be considerable challenges faced in producing enough high-quality protein to sustain the growing population, without further increasing the pressure on our natural resources. As it stands, the EU relies heavily on imported protein sources (Rostoks *et al.,* 2019), including soybeans, for food and feed leaving Europe at risk of a protein gap. As such, there is a now a pressing need for the EU to find new sustainable protein sources that are high quality, healthy and have the desired functional properties that meet consumer needs and expectations. The InnoProtein project seeks to promote protein self-sufficiency in Europe by investigating the application of novel sustainable protein sources. The project aims to examine the suitability of Single Cell Proteins (SCPs) including bacteria, microalgae, and fungi, as well as entomological alternatives for use as high-quality proteins for food, feed, and non-food biobased applications.

However, there are certain requirements that must be taken into account when considering these new sustainable protein sources for food, feed, and non-food biobased applications. The first of these being the social requirements. With regards to food applications the health and nutritional requirements of the general population need to be established, assessing the needs of the population at various life stages, with particular attention to their amino acid requirements. Similarly, in the case of feed applications the same requirements will also be considered for pigs, poultry, and trout. Furthermore, the toxicological safety limits of these proteins must be looked at, including heavy metal contamination, microbiological assessment and allergenicity. As well as this there are a number of other factors relating to these proteins that must be considered, including their sensorial, functional, and physical characteristics.

Another aspect that must be considered are the market requirements for these protein sources. It is becoming increasingly evident that the current global landscape is leading to a demand for new sustainable food sources, particularly new and alternative proteins. However, given the unconventional nature of the proteins being investigated in the InnoProtein project there are a number of factors that must be analysed when contemplating their introduction to the market. The current market value of SCPs and entomological proteins must be assessed, as well as their projected value over the coming years. It is also important to consider the barriers to market entry for these proteins, and to identify strategies to overcome such barriers, allowing for their successful entry to market.

Furthermore, the environmental requirements for the InnoProtein project must also be investigated. Along with the growing population the world is in the midst of a climate crisis and is facing a significant depletion of its natural resources. With this in mind, we must now shift our focus from intensive production to more sustainable, environmentally safe practices when producing new sustainable proteins for the InnoProtein project. It is the hope that these new sustainable practices will free up land, reduce water use and significantly reduce greenhouse gas emissions (GHGs).

Finally, in order to ensure that InnoProtein proteins are produced to a high standard and are safe for these proposed end user applications the regulatory requirements must be assessed. By identifying these regulations and standards in the early stages of the InnoProtein project we can make certain these protein products are safe for the end users and that their production is consistent throughout. Therefore, greatly increasing the possibility that these proteins will make it to market. With this in mind an in-depth inventory of regulations, standards, and guidelines, gathered from national, EU and international databases is to be compiled. It is the aim of this inventory to guide all involved in the InnoProtein project as they conduct their work, providing them with the necessary regulatory information to ensure total compliance throughout the project.

The purpose of Deliverable D1.1 is to set down the sustainable requirements, i.e., social, market, environmental, and regulatory, that are needed for the InnoProtein project to enable the unconventional protein value chain to meet end user expectations.

1. Task 1.1. Social Requirements

1.1. Introduction

By 2050, it is predicted that there will be upwards of 9.8 billion inhabitants worldwide. As such, our greatest environmental, economic, and social challenges will be to produce enough quality protein in a sustainable manner. Thereby reducing the pressure on current natural resources in the production of food and feed. In this context, the EU is vulnerable due to a protein gap as it relies significantly on the importation of protein sources, importing 70% of protein-rich crops and 85% of soybeans (Rostoks *et al*., 2019). Therefore, there is now an urgent need for new sustainable protein sources with high nutritional quality and functional properties that meet end-users' needs and expectations in food, feed, and non-food biobased markets. The InnoProtein project fully faces this challenge and fits into the Farm to Fork Strategy and the European Green Deal priorities addressing unexploitable and sustainable protein sources to accelerate Europe's protein self-sufficiency. The project aims to provide single cell proteins (microalgae, bacteria & fungal) and entomological alternative high-quality proteins for food, feed, and non-food biobased applications with a circular and zero-waste perspective.

Task 1.1. will cover the social requirements for food and feed applications, hereunder the health and nutritional requirements. In food applications, the amino acid requirements of the general population will be assessed, considering several growth stages. For feed applications, the amino acid requirements for pig, poultry and trout will be established. In both food and feed applications, the toxicological safety limits, sensory and physical properties, functionalisation, and purity required for the final InnoProtein protein products to meet customers' expectations will be defined. Finally, the requirements for non-food bio-based applications will be established.

These social requirements will be essential for single-cell protein (SCP) and insect protein producers to consider from the beginning of the project to carry out their tasks under the subsequent work packages 2-6.

1.2. Food applications

1.2.1. Protein requirements

Proteins are large, complex molecules made up of chains of smaller units called amino acids. There are 20 different types of amino acids that can be combined in various sequences to form a wide variety of proteins. These amino acids are linked together by peptide bonds, creating long chains with specific structures and functions (Ross *et al*., 2013). Virtually all biological processes require proteins, as proteins are structural components in cells and tissues, act as enzymes to catalyse chemical reactions, participate in cell signalling, facilitate transport of molecules, and contribute to the defence mechanisms of the immune system, among many other functions (Nelson & Cox, 2017). Therefore, proteins are essential macronutrients in the human diet as a source of amino acids. They can be obtained from a range of food sources, including animal and plant-based products (Gropper *et al*., 2017).

Protein requirements vary based on factors such as age, gender, activity level, and specific health conditions. Below is an overview of the human protein requirements divided into different age and life stages, concluding with a summary table. The recommendations are in agreement with the latest scientific opinion on dietary reference values for protein by the European Food Safety Authority (EFSA), which considers recommendations by among others the Word Health Organisation (WHO), the Food and Agricultural Organization (FAO), the United Nations University (UNU), the Nordic Nutrition Recommendations (NNR), the German Nutrition Society (DGE/D-A-CH), and the French Agency for Food Safety (AFSSA).

Healthy adults

Based on the available evidence of nitrogen balance and isotope tracer studies, the average requirement (AR) and recommended intake (RI) for healthy adults have been set to 0.66 g/kg and 0.83 g/kg body weight per day, respectively, in the scientific opinion by EFSA (2012). The recommendations stem from a meta-analysis of nitrogen balance studies in humans by Rand *et al*. (2003), which involved studies stratified for subpopulations, settings in different climates, sex, age, and protein sources, resulting in a recommended average requirement of 0.66 g/kg body weight per day as the best estimate. The recommended intake (RI) has been identified as the 97.5th percentile of the population distribution of requirement, which corresponds to 0.83 g/kg body weight per day of protein (WHO/FAO/UNU, 2007).

This protein intake should also adequately meet the requirements for essential amino acids. The recommended intake range is 10–20 E% of total energy intake. With an energy intake below approximately 8 MJ or 1,900 kcal (e.g., at low body weight, having low physical activity levels, or during intentional weight loss), the protein E% should be increased correspondingly to ensure that the AR and RI is met (Blomhoff *et al*., 2023).

Older adults

There is contradictory data available on the protein requirement of older adults compared to younger adults. In its scientific opinion, EFSA (2012) concludes that the available data are insufficient to determine the protein requirement in older adults and that at least the same level of protein intake for young adults is required for older adults. Thus, they recommend the same AR (0.66 g/kg) and RI (0.83 g/kg) based on nitrogen balance for older adults (EFSA, 2012). However, the low energy requirement of sedentary elderly people means that the protein to energy ratio of their requirement is higher than for younger, more active age adults.

Indeed, recent studies have found that intakes above the RI may be optimal to prevent decline of physical functioning, pointing towards a recommended range for older adults of 1.2–1.5 g/kg body weight, approximately 15–20 E% (Geirsdóttir & Pajari, 2023).

Pregnant and lactating individuals

Studies show that protein is not deposited equally throughout pregnancy, but that protein deposition in maternal and foetal tissues increases, with most occurring during the third trimester (Rajavel & Ball, 2016). Additional protein of 1, 9 and 31 g protein/day in the first, second and third trimesters, respectively, is recommended for pregnant women (EFSA, 2012).

During lactation, an additional protein intake of 19 g/day is recommended during the first three months, falling to 12.5 g/day after 6 months. This is based on the increased nitrogen needs of lactating women in order to synthesize milk proteins and assumes that the efficiency of milk protein production is the same as the efficiency of protein synthesis in non-lactating adults (EFSA, 2012).

Children and adolescents

The protein requirements of infants and children includes two components: maintenance requirement and growth requirement. This can be defined as the minimum intake that allows a positive nitrogen equilibrium to facilitate growth in normally growing subjects who have an appropriate body composition, are in energy balance, and are moderately physically active. The recommended intakes fall promptly in the first two years of life (RI at 0.5 years of age is 1.31 g/kg per day; RI at two years of age is 0.97 g/kg per day). Thereafter, the decrease towards the adult level is very slow (WHO/FAO/UNU, 2007). For young children below 2 years of age, it is advisable not to exceed a range of 10–15 E% protein (Blomhoff *et al*., 2023).

Protein recommendations overview

Table 1 below presents an overview of the protein recommendations as outlined above.

Table 1. Human Protein requirements

Age (years), or life stage	AR (g/kg/d[1])	RI (g/kg/d[1])	Reference weight (kg)[1] Males	Reference weight (kg)[1] Females	RI (g/d) Males	RI (g/d) Females
0.5	1.12	1.31	7.7	7.1	10	9
1	0.95	1.14	10.2	9.5	12	11
1.5	0.85	1.03	11.6	10.9	12	11
2	0.79	0.97	12.7	12.1	12	12
3	0.73	0.90	14.7	14.2	13	12
4	0.69	0.86	17.0	16.4	15	14
5	0.69	0.85	19.2	18.7	16	16
6	0.72	0.89	21.5	21.1	19	19
7	0.74	0.91	24.3	23.8	22	22
8	0.75	0.92	27.4	26.8	25	25
9	0.75	0.92	30.6	30.0	28	28
10	0.75	0.91	33.8	33.7	31	31
11	0.75 (m), 0.73 (f)	0.91 (m), 0.90 (f)	37.3	37.9	34	34
12	0.74 (m), 0.72 (f)	0.90 (m), 0.89 (f)	41.5	42.6	37	38
13	0.73 (m), 0.71 (f)	0.90 (m), 0.88 (f)	46.7	47.5	42	42
14	0.72 (m), 0.70 (f)	0.89 (m), 0.87 (f)	52.7	51.6	47	45
15	0.72 (m), 0.69 (f)	0.88 (m), 0.85 (f)	59.0	54.6	52	46
16	0.71 (m), 0.68 (f)	0.87 (m), 0.84 (f)	64.1	56.4	56	47
17	0.70 (m), 0.67)	0.86 (m), 0.83 (f)	67.5	57.4	58	48
18-59	0.66	0.83	74.6	62.1	62	52
≥ 60[3]	0.66	0.83	73.5	66.1	61	55
Pregnant women[2]						
1st trimester	-	-	-	-	-	+1
2nd trimester	-	-	-	-	-	+9
3rd trimester	-	-	-	-	-	+28
Lactating women[2]						
0-6 months	-	-	-	-	-	+19
>6 months post-partum	-	-	-	-	-	+13

[1] For infants and children, based upon the 50th percentile of the reference body weights (kg) of European children (van Buuren et al., 2012). For adults, based upon weighted median body weights (kg) of European men and women (SCF, 1993)
[2] In addition to the PRI for non-pregnant women
[3] Recent studies point towards a recommended range for older adults of 1.2–1.5 g/kg body weight (Geirsdóttir & Pajari, 2023).
AR= Average requirement. RI = Recommended intake

1.2.2. Amino acid requirements

Amino acid requirements for humans

As mentioned in above chapter, amino acids are the building blocks of proteins. There are hundreds of amino acids in nature, but only 20 of these are used to build proteins in living organisms (Erdman *et al.*, 2012). These amino acids each have specific metabolic roles in the human body that take place as they are liberated after absorption of dietary protein; some are oxidised for energy, some are incorporated into protein in the body, while some are used to form other nitrogen-containing compounds (Ross *et al.*, 2013).

Of these 20 amino acids, some are synthesized de novo in the body and are thus called nonessential or dispensable amino acids. Essential or indispensable amino acids cannot be synthesised by the human body and must therefore be provided in the diet (Ross *et al.*, 2013). The 9 indispensable amino acids are: leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine.

The protein requirement is dependent on the dietary protein quality, which is mainly determined by the pattern of essential amino acids in the protein. Dietary proteins of animal origin or a combination of plant proteins from, for example, legumes and cereal grains, have a good distribution of essential amino acids (Geirsdóttir & Pajari, 2023).

Making recommendations for the intake of each indispensable amino acid is complicated since amino acids are not provided as individual nutrients in the diet but in the form of protein. Thus, it should be noted that the values for indispensable amino acid requirements are not yet sufficiently precise.

There are a number of different approaches that can and have been used to assess indispensable amino acid requirements. These requirements were first determined in adults using a pioneering nitrogen balance approach (Rose, 1957), but this approach has since thought to underestimate the requirements (Rand and Young, 1999; WHO/FAO/UNU, 2007; Young and Marchini, 1990). More recent data have been obtained using amino acid labelling with stable isotopes, which is based on the measurement of amino acid oxidation as a function of intake (Bos *et al.*, 2002). This includes the indicator amino acid balance method (Young and Borgonha, 2000), the indicator amino acid oxidation method (Elango *et al.*, 2008a, 2008b; Pencharz and Ball, 2003), the 24h-indicator amino acid oxidation method (Kurpad *et al.*, 2001), and the protein post-prandial retention method (Bos *et al.*, 2005; Millward *et al.*, 2000).

The amino acid requirements of children have been made using a factorial method, based on the estimated protein requirements for maintenance and growth (WHO/FAO/UNU, 2007). In this method, it is assumed that the required amino acid composition for maintenance is the same as for adults, and that the amino acid pattern required for growth is determined by the amino acid composition of whole-body tissue protein. The mean values for indispensable amino acid requirements for children and adults are provided in Table 2. These recommendations are derived from the scientific opinion by EFSA (2012).

Table 2. Mean requirements for indispensable amino acids in children and adults

Indispensable amino acids (mg/kg/d)	0.5 y	1-2 y	3-10 y	11-14 y	15-18 y	Adults >18 y
Histidine	22	15	12	12	11	10
Isoleucine	36	27	23	22	21	20
Leucine	73	54	44	44	42	39
Lysine	64	45	35	35	33	30
Methionine + cysteine	31	22	18	17	16	15
Phenylalanine+ tyrosine	59	40	30	30	28	25
Threonine	34	23	18	18	17	15
Tryptophan	9.5	6.4	4.8	4.8	4.5	4
Valine	49	36	29	29	28	26

Amino acid content of quality protein

The quality of protein is defined by its ability to support growth. As such, high-quality protein produces a faster growth rate. One method to evaluate the quality of protein is to assess the reference pattern of amino acids (mg/g protein) (Ross *et al.*, 2013; WHO/FAO/UNU, 2007). This is done by dividing the requirement (mg amino acid/kg body weight per day) by the average requirement for protein (g/kg body weight per day). Age-specific patterns for dietary proteins can be calculated by dividing the requirement of each indispensable amino acid by the protein requirement of the specific age group (WHO/FAO/UNU, 2007) (Table 3). The reference pattern of amino acids for infants <0.5 years is the amino acid pattern of human milk.

Table 3. Scoring pattern for indispensable amino acids for children and adults

Recommended mg amino acid per g protein (mg/g)	0.5 y	1-2 y	3-10 y	11-14 y	15-18 y	Adults >18 y
Histidine	20	18	16	16	16	15
Isoleucine	32	31	31	30	30	30
Leucine	66	63	61	60	60	59
Lysine	57	52	48	48	47	45
Methionine+ cysteine	28	26	24	23	23	22
Phenylalanine+ tyrosine	52	46	41	41	40	38
Threonine	31	27	25	25	24	23
Tryptophan	8.5	7.4	6.6	6.5	6.3	6
Valine	43	42	40	40	40	39

1.2.3. Toxicological safety limits for food

The safety assessments of the InnoProtein samples for incorporation into foodstuff will be performed by testing the presence of environmental contaminants including heavy metals (such as copper, cadmium, mercury, arsenic, and lead), pesticides, and potential pathogens. Consumers are mainly exposed to the residues of pesticides and heavy metals through their dietary intake (Akande & Akande, 2021).

Heavy metals

The concentrations of mercury (Hg), copper (Cu), cadmium (Cd), arsenic (As) and lead (Pb) will be compared to the limits defined by Codex Alimentarius, which will vary depending on the InnoProtein material assessed. For example, the maximum level (ML) of Cd under the category of 'legume vegetables' was specified as 100ng/g, and for Pb the ML was 100ng/g. The provisional tolerable weekly intake (PTWI) of As was 3.0 μg/kg bw/day, and the PTWI for inorganic Mercury was 4 μg/kg bw.

Pesticide residues

Samples will be assessed for pesticide residues above the maximum residue level (MRL) set by the EU. MRLs are the highest concentrations of pesticide residues that, in accordance with good agricultural practice (GAP) and the lowest exposure necessary to protect vulnerable consumers, are legally acceptable in or on food or animal feed. MRLs have been set for more than 500 pesticides and these will be documented for the relevant InnoProtein samples.

Microbial assessment

Since bacteria are present in all types of water, food, soil, vegetation, and air (Allen *et al*., 2004), microbiological evaluation of the InnoProtein samples is necessary to identify the presence of any spoilage or pathogenic microorganisms. For total microbial levels in raw foods samples containing less than 10^4 cfu/g, organisms can be qualified as "good" and only those having more than 5 X 10^7 organisms are qualified as "spoiled food". Samples should be free from pathogens such as coliforms, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and Enterobacteriaceae.

Cell toxicity

For measuring the potential toxicity of the InnoProtein samples, standard in vitro toxicity screening will be performed using the Ames mutagenesis assay. The potential cytotoxic effects of the samples will be determined by the MTT and Alamar blue assay against a dose-viability curve to evaluate the possible toxicity at high doses. For assessing any genotoxic risk, Comet assay (or Single Cell Gel Electrophoresis assay) will be performed. All samples should be non-mutagenic and have no cytotoxic or genotoxic effect at the levels utilised in the final formulations.

Allergens

In the EU, there are 14 food ingredients that have been declared as allergens (celery, cereals containing gluten, crustaceans, eggs, fish, lupin, milk, molluscs, mustard, peanuts, sesame, soybeans, sulphur dioxide, and sulphites). Therefore, the composition of a substance should be appropriately labelled, wherein, a proven allergenic or intolerant effect is known to occur in consumers. Allergenicity studies will be performed to identify the presence of any allergens in the InnoProtein samples.

Antinutritional compounds

Antinutritional compounds that can exist in extracts include phytic acid, trypsin inhibitors, amylase inhibitors, proteases, tannins, saponins and lectins. The presence and level of these compounds in the relevant InnoProtein samples will be monitored. Nucleic acid content in the SCP samples will also be monitored.

1.2.4. Sensory and physical properties

Proteins intended for use in food applications must meet specific sensory and physical requirements to ensure their suitability. These requirements encompass sensory quality, physical properties, and nutritional aspects. In summary, proteins for food applications must meet specific sensory, physical, and nutritional requirements to ensure they are suitable for use in various food products, providing both functional benefits and contributing to the overall quality and nutritional value of the final products. Below is a comprehensive overview of the internal needs and prerequisites for proteins in food applications.

Physical properties

- Physical form: The protein should be in the form of a powder, which is convenient for mixing, blending, and dispersing in different food preparations.
- Solubility: The protein must be highly soluble in water, without forming lumps or introducing any off-colours into the solution. This ensures that it can be easily incorporated into a wide range of food and beverage products.
- pH: Ideally, the protein should have a neutral pH, which is compatible with many food systems and minimises the need for extensive pH adjustments during product formulation.

Sensory quality appearance

- Colour: The protein should appear as a white to slightly yellow or beige powder. It should not have a dusty or clumpy texture, ensuring ease of handling and incorporation into food products.
- Smell: The protein should be odourless or have a neutral smell, with no off-putting odours that could negatively impact the overall flavour of the food product.
- Taste: The protein should have a neutral taste, avoiding of any off-tastes. In some cases, the protein owns the characteristic taste of the original source, which is acceptable if it complements the final product.

Nutritional properties

- Protein content: The protein product should have a high protein content, more than 80% if possible. A high protein content is desirable to enhance the nutritional value of the final food product.
- Amino acid profile: The protein should contain a full complement of amino acids, ensuring that it provides a balanced source of essential amino acids. The higher the amount of different amino acids (especially the essential amino acids), the

higher the quality of the product. If a protein does not contain a full profile of amino acids, combining various protein sources is a strategic approach to enhance the overall amino acid profile in a vegan diet. Each plant has an unique set of amino acids and by blending sources, a more comprehensive and balanced intake of essential amino acids is ensured.

- Other macronutrients: Besides protein, the protein source should not introduce undesirable levels of other macronutrients, such as fats, carbohydrates, and sugars. These should be minimal to avoid interference with the intended nutritional profile of the final product.
- Anti-nutritional factors: The presence of anti-nutritional factors, such as phytates, tannins, protease inhibitors, and similar compounds, should be minimised. Phytates can form complexes with e.g. calcium, iron and zinc, which then minimizes the bioavailability of these minerals. Tannins can form complexes with iron, which also reduces the bioavailability of this mineral. Protease inhibitors interfere with the activity of protease, which is responsible for breaking down proteins during digestion, which then can result in hindering the absorption of amino acids. Reducing the content of these factors is essential to enhance the bioavailability of nutrients and improve the overall nutritional quality of the food product.

1.2.5. Functionalisation and purity

Functionalisation

Powder-based standard applications typically require proteins that are as neutral as possible, devoid of characteristics such as thickening, emulsifying, or gelling properties, which could influence the desired end result of a product. However, the specific application can significantly influence the desired protein properties. Thus, when a powder mixture contains fatty raw materials (e.g. fat powders) or when an oil-in-powder mixture is required, emulsifying properties could be desired and helpful. Furthermore, in the context of a powder blend developed for food gelling processes, proteins containing gelling properties could potentially reduce the need for additional (or already implemented) gelling agents which could possibly lower the price of the end product. Or if a protein shows thickening properties, it could be incorporated into ready-to-mix soups to increase viscosity. Thus, the relevance of specific protein's properties varies depending on the intended application, or conversely, the product development should be based on the specific proteins characteristics to meet specific functional requirements.

Purity

Purity is a critical factor when it comes to proteins used in food applications. Ensuring high purity is important for several reasons:

- Safety: Purity is directly linked to the safety of the food product. Contaminants or impurities can cause health issues to consumers. To guarantee the safety of the final food product, the protein source has to be free from substances such as heavy metals, microbial contaminants, and chemical residues.
- Consistency: Consistent purity is essential for consistent quality in food products. Varying levels of impurities can lead to inconsistent taste, texture, and appearance of the final food product. Consistency is crucial to meet consumer expectations and brand reputation.
- Flavour and aroma: Unwanted impurities can cause off-flavours and odours to the food product. Pure proteins are able to allow the natural and intended flavours and aromas of the food to shine through, ensuring a pleasing sensory experience for consumers.
- Functionality: Purity affects the functional properties of proteins. In food applications, proteins are sometimes used for their emulsifying, gelling, or stabilizing properties. Impurities can disrupt these functionalities, leading to product failure.
- Labelling and regulation: Many countries have strict regulations regarding food safety and labelling. Impurities or contaminants may result in non-compliance with these regulations, leading to legal issues and product recalls.

Regular monitoring and testing should be carried out at different stages of production to verify the absence of contaminants. Meeting purity standards not only safeguards the health and satisfaction of consumers but also upholds the integrity of the food industry.

1.3. Feed applications – for poultry, pigs and trout

1.3.1. Amino acid requirements for pigs, poultry, and trout

Proteins are an essential component of the animal diet, needed for growth, development, reproduction, and survival. In the animal body, proteins are the primary constituent of structural and protective tissues (e.g., bones, ligaments, scales, and skin), soft tissues (organs, muscle) and body fluids. If a diet contains an inadequate amount of protein, there will be a reduction or cessation of growth. Table 4 presents the percentages of different body components and the percentage of the amino acids of this body components in pigs.

Table 4. Amino acid composition of body components and milk in pigs

Element	Body components				
	Skeleton	Offal	Blood	Hair	Leche
Contribution (%)					
At 8.5 kg	66.2	28.0	4.0	1.8	
At 107 kg	78.8	14.1	5.4	1.7	
Amino acids (%)					
Lys	7.6	6.6	9.0	4.0	7.4
Met	1.9	1.6	0.8	0.5	2.0
Cys	1.1	1.3	1.5	13.0	1.7
Thr	4.0	3.6	3.7	5.7	4.3
Trp	1.1	1.3	1.5	0.3	1.4
Val	4.7	4.9	9.0	5.9	5.1
Ile	3.9	3.5	1.3	3.7	4.3
Leu	7.1	7.1	13.0	8.0	8.7
Phe	3.8	4.0	6.8	2.7	4.2
Tyr	3.0	3.1	2.9	3.4	4.1
His	3.7	2.8	5.6	2.0	3.9
Arg	6.5	5.6	3.8	6.5	5.5

Source: Van milgen and Dourmad (2015).

For the feed formulation of farmed animals, amino acids are more important than protein, but given the lack of information on the actual amino acid content of some non-traditional raw materials and to prevent the lack of any amino acid from limiting animal growth, a minimum amount of protein is included in the feed.

Dietary protein and amino acids are major factors influencing the productivity of farmed animals. Amino acids provide essential nitrogen for the synthesis of protein (muscle growth) and other biological molecules (development of foetuses and supporting tissues, milk production, etc.).

There are about 22 or more amino acids that forms the building blocks for all complex proteins. Therefore, dietary requirement for protein is essentially a requirement of the amino acids contained in the protein. The composition of commercial diets is generally described in terms of protein concentration. However, the protein quality in terms of the nutritional value of diets depends on its amino acid content and on physiological utilisation of specific amino acids after digestion, absorption, and a minimal rate of oxidation. Availability of amino acids varies with protein source, processing treatment, and interaction with other components of the diet.

However, the nutritional classification of amino acids for terrestrial animals is divided into three categories based on their absolute or relative rates of protein synthesis in vivo:

- Indispensable (essential): histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.
- Conditionally dispensable: arginine, cysteine, tyrosine
- Dispensable (non-essential): alanine, aspartic acid, asparagine, glutamic acid, glutamine, glycine, proline, and serine.

The terms indispensable (essential) and dispensable (non-essential) are widely used to classify the nutritional importance of amino acids in animal feed. The ten essential or indispensable amino acids cannot be synthesized by animals, and therefore must be provided in the diet. The metabolism and requirement of certain conditionally dispensable amino acids may vary among animals including fish (D'Mello, 2003).

Amino acids requirements for pigs

Pigs, like other animals, require a number of essential amino acids in their diet to maintain proper growth and development. Amino acid needs in pigs can vary depending on the growth stage and the purpose of breeding (e.g., breeding for meat or breeding). Mentioned below are some of the essential amino acids in the pig diet:

- Lysine: Lysine is one of the most critical essential amino acids for growing pigs. Lysine requirements vary by growth stage and can range from 0.9% to 1.1% in the diet, depending on factors such as weight and growth rate.
- Methionine: Methionine is another essential amino acid important for the growth and health of pigs. Methionine requirements are typically around 0.25% in the diet.
- Threonine: Threonine is necessary for optimal growth and development. Threonine requirements are usually in the range of 0.65% to 0.75% in the diet.
- Tryptophan: Tryptophan is an essential amino acid that influences the appetite and behaviour of pigs. Tryptophan requirements are generally around 0.18% in the diet.
- Valine, isoleucine, and leucine: These three essential amino acids, known as branched-chain amino acids, are essential for muscle growth and protein production. Requirements for these amino acids vary but are generally in the range of 0.75% to 1.0% in the diet.

The amino acids of greatest practical importance in diet formulation (i.e., those most likely to be at deficient levels) are lysine, tryptophan, threonine, and methionine. Corn, the basic grain in most swine diets, is markedly deficient in lysine and tryptophan. The other principal grains for pigs (grain sorghum, barley, and wheat) are low in lysine and threonine. The first limiting amino acid in soybean meal is methionine, but sufficient amounts are provided when soybean meal is combined with cereal grains into a complete diet that meets the lysine requirement. An exception might be in young pigs that consume diets with high levels of soybean meal or diets containing dried blood products low in the sulphur-amino acids.

Milk protein is well balanced in essential amino acids but is usually too expensive to be used in swine diets, except for very young pigs. Dried whey, commonly used in starter diets, contains protein with an excellent profile of amino acids, but the total protein content of whey is low. Diets based on corn and animal-protein byproducts (e.g., meat meal, meat, and bone meal mix) are inferior to corn-soybean meal diets, but they can be improved significantly by adding tryptophan or supplements that are good sources of tryptophan. Animal proteins are also good sources of minerals and B-complex vitamins.

Diets formulated for early weaned pigs that contain high levels of dried animal plasma or dried blood cells may be deficient in methionine. However, high levels of methionine can depress growth, so methionine should not be added indiscriminately to diets. Supplemental valine may be of value in corn-soybean meal diets fed to lactating sows, but it is still too expensive to be considered as a dietary supplement (Cromwell, 2002).

The amino acid requirements of piglets and fattening pigs have been studied in detail. In general, it is recommended to formulate feeds based on "ideal protein" (Mitchell, 1964), particularly interesting when substantial amounts of byproduct feed are included in the diet. It refers to a situation where all essential amino acids are co-limiting for performance so that the amino acids supply exactly matches its necessity. The requirements for amino acids in "ideal protein" are usually expressed relative to the requirement for lysine (i.e., Lys = 100%). The expression relative to lysine is very useful from a practical point of view. Lysine is typically the first-limiting amino acid in diets for pigs. Lysine has therefore received most of the nutritionists' attention and considerable research has been carried out to describe the change in lysine requirements during growth, gestation, and lactation. The following table 5 provides recommended values to use on the "ideal protein" in pigs according to the productive status according to studies by BSAS (2003), FEDNA (2006), NRC (2012) and National Swine Nutrition Guide (2010).

Table 5. Ideal protein in pigs (% in relation to lysine requirements)

	Gestation	Lactation	Piglets 5-20 kg	Growth 20-100 kg	Finishing >100 kg
Lys	100	100	100	100	100
Met	31	30	30	31	32
Met+Cys	66	56	59	60	61
Thr	72	66	65	65	66
Trp	19	19	20	19	19
Ile	60	57	54	55	56
Leu	98	113	100	100	100
Val	70	80	69	68	67
His	35	40	32	33	34
Phe	57	56	56	60	61
Phe+Tyr	100	113	97	99	99
Arg	72	62	42	42	43

Source: FEDNA (2013).

It is important to note that specific amino acid needs can vary depending on the pig's genetics, the quality of the ingredients in the diet, and other factors.

Amino acids requirements for poultry

In the feeding of poultry, proteins can be replaced by digestible amino acids in their diet since these are needed for their growth and production. If data are available on the amount of digestible amino acids in the composition of ingredients and the needs of

poultry, this can facilitate the reduction of the amount of protein in the diet (Siegert and Rodehutscord, 2017; Belloir *et al*., 2017).
Amino acid needs in poultry farming are critical for optimal growth and development. Ensuring that broilers and laying hens receive the right amount of digestible essential amino acids in their diet is crucial to achieving an economic return in chicken meat and eggs production. To maintain optimal yields, the ratio of essential to non-essential amino acids should be around 55:45 (Bedford and Summers, 1985).

Mentioned below are some of the key essential amino acids and their requirements in the broilers and laying hens' diet:

- Lysine: Lysine is the most critical essential amino acid in broiler feed. In laying hens, it is an essential amino acid for the production of good quality eggs and feather growth. Lysine requirements vary by growth stage and can range from 1.0% to 1.3% in the diet, depending on the genotype of the chicken and hen as well as other factors.
- Methionine: Methionine is another essential amino acid important for broiler growth and health. It is an important amino acid that is key to the production of egg protein and the proper development of the skin and feathers of hens. Methionine requirements are around 0.45% in the diet.
- Threonine: Threonine is necessary for optimal growth and muscle protein production in both broilers and laying hens. Threonine requirements are usually between 0.70% and 0.80% of the diet.
- Tryptophan: Tryptophan is necessary for amino acid balance and bird welfare. It is also necessary for the conversion of niacin (essential nutrient). Tryptophan requirements are generally around 0.20% in the diet.
- Valine, isoleucine, and leucine: These three essential amino acids, known as branched-chain amino acids, are essential for muscle growth and the regulation of protein synthesis. Requirements for these amino acids vary but are generally in the range of 0.80% to 1.0% in the diet.
- Arginine, histidine, and phenylalanine: These amino acids are also essential and should be present in the diet in adequate amounts.
- Phenylalanine is necessary for the production of methanin, which is necessary for feather pigmentation and to maintain good feather health.

The formulation of diets based on the "ideal protein" helps to define the digestible amino acid needs of birds (Emmeret & Baker, 1997). The system is based on determining the lysine needs of birds and using a percentage or fixed ratio with respect to lysine for the rest of the essential amino acids according to each stage of development, thus avoiding excess nitrogen. Table 6 provides recommended values to use for the "ideal protein" in broilers according to the productive status according to different studies by Baker and Han (1994), Baker (2009), Coon (2004), Samadi and Liebert (2008), Corzo et al. (2011) and Dozier and Gehring (2014).

Table 6. Ideal protein in broilers, Digestible amino acids (% in relation to Lysine requirements)

	Beginning (0-21 d)	Finishing (21d to sacrifice)
Lys	100	100
Met	40	41
Met+Cys	74	76
Thr	65	66
Trp	17	18
Ile	67	68
Leu	107	107
Val	78.5	79
Phe+Tyr	100	113
Arg	105	103

Source: FEDNA (2018).

Table 7 provides recommended values to use for the ideal protein in laying hens according to the productive status according to different studies by De Blas y Mateos (1991), NRC (2012), Fefana (2014), Van Krimpen (2015) and Rostagno *et al*. (2017), and the standards of genetics and additives companies.

Table 7. Ideal protein in chicks and lying hens. Digestible amino acids (% in relation to lysine requirements)

	Breeding chicks			Laying hens	Males
	0-5 weeks	5-10 weeks	10-17 weeks	≥ 17 weeks	
Lys	100	100	100	100	100
Met	44	45	47	50	40
Met+Cys	75	80	84	88	80
Thr	66	68	72	70	24
Trp	19	20	22	21	80
Ile	68	70	71	80	49
Leu	107	109	110	115	109
Val	75	76	77	89	78
Arg	105	105	106	104	105

[1]Glycine Equivalent = Gly (%) + 0.7143 Ser (%). Source: FEDNA (2018).

It is important to remember that specific amino acid requirements can vary depending on the genetics of the chickens and hens, the quality of the ingredients in the diet, and other factors. Broiler producers often use diet formulation programs to calculate and adjust amino acid levels in feed according to the needs of birds at different stages of growth.

Amino acid requirements for trout

The amino acid requirements in the diet of rainbow trout (Oncorhynchus mykiss) vary depending on its developmental stage, size, and culture conditions. Essential amino acids are vital for the growth, health, and reproduction of these fish. Here is a summary of some of the key essential amino acids and their requirements in the rainbow trout diet:

- Lysine: Lysine is an essential amino acid important for the growth and health of rainbow trout. Lysine requirements can vary but are generally in the range of 1.0% to 2.0% in the diet, depending on the stage of growth and other factors.
- Methionine: Methionine is crucial for protein synthesis and growth. Methionine requirements are usually in the range of 0.5% to 1.5% in the diet.
- Threonine: Threonine is necessary for protein production and muscle growth. Threonine requirements vary and are generally in the range of 0.6% to 1.0% in the diet.
- Tryptophan: Tryptophan is an essential amino acid that influences metabolism and stress response in fish. Tryptophan requirements can be in the range of 0.2% to 0.5% in the diet.
- Valine, isoleucine, and leucine: These branched-chain amino acids (BCAAs) are essential for protein synthesis and muscle growth. Requirements for these amino acids vary but are generally in the range of 1.0% to 3.0% of the diet.
- Arginine, histidine, phenylalanine, and other amino acids are also important in the diet of rainbow trout.

It is important to remember that amino acid requirements can vary depending on the quality of the ingredients in the diet and the growing conditions. Table 8 provides essential amino acids requirement of rainbow trout listed as % of protein.

Table 8. Essential amino acids requirements of rainbow trout (values are the average of several scientific papers)

	Rainbow trout
Lys	4.8 [1,2,3,4,5]
Met	2.2 [1,4,6]
Cys	0.2 [1,4,6]
Thr	3.4 [4,7]
Trp	0.8 [4,8, 16]
Ile	2.4 [4]
Leu	4.4 [4]
Val	3.1 [4]
His	1.6 [4]
Phe+Tyr	4.8 [9]
Arg	4.3 [1,5,7,10,11,12,13,14,15]

Source: Lall et al., (2015)

[1] Kim *et al.* (1992); [2] Walton *et al.* (1984);[3] Pfeffer *et al.* (1992); [4] Ogino (1980); [5] Ketola (1983); [6] Walton *et al.* (1984); [7] Rodehutscord *et al.* (1995); [8] Poston and Rumsey (1983); [9] Kim *et al.* (1993); [10] Kaushik and Luquet (1979); [11] Akiyama *et al.* (1985); [12] Foster (1993); [13] Walton *et al.* (1986); [14] Pack *et al.* (1995); [15] Cho *et al.* (1992); [16] Kim *et al.* (1987)

1.3.2. Toxicological safety limits for feed

The safety assessments of the InnoProtein samples for incorporation into animal feed will be performed by testing the presence of environmental contaminants including heavy metals (such as copper, cadmium, mercury, arsenic, and lead), pesticides, and potential pathogens. Animals are mainly exposed to the residues of pesticides and heavy metals mostly through the plants, feed, soil, and water in their environment (Amadi *et al.*, 2022)

Heavy metals

The concentrations of mercury (Hg), copper (Cu), cadmium (Cd), arsenic (As) and lead (Pb) will be compared to reported maximum level (ML) limits, which will vary depending on the InnoProtein material assessed. The tolerable intake of heavy metals by animals varies by animal, breed, and husbandry (Tahir and Ali Alkheraije, 2023).

Pesticide residues

Maximum residue limits/levels (MRLs) are specified under EU and national regulations for approved veterinary medicines and pesticides, and maximum levels (MLs) are similarly specified for contaminants in foods of animal origin. Samples will be assessed for pesticide residues above the maximum residue level (MRL) set by the EU. MRLs are the highest concentrations of pesticide residues that, in accordance with good agricultural practice (GAP) and the lowest exposure necessary to protect vulnerable consumers, are legally acceptable in or on food or animal feed.

Microbial assessment

Since bacteria are present in all types of water, food, soil, vegetation, and air (Allen *et al*., 2004), microbiological evaluation of the InnoProtein samples is necessary to identify the presence of any spoilage or pathogenic microorganisms. According to Kukier *et al*. (2012) about the microbiological quality of livestock feed, total aerobic microbial count (TAMC) should not surpass 10^6 cfu/g. Samples should be free from pathogens such as coliforms, *Salmonella, Escherichia coli, Staphylococcus aureus* and Enterobacteriaceae.

Allergens

In the EU, the 14 food ingredients that have been declared as allergens for humans and do not apply to animal feed. Ingredients of animal feed will vary depending on the animal being fed with new proteins should be monitored for potential allergenicity response.

Antinutritional compounds

Antinutritional compounds that can exist in extracts include phytic acid, trypsin inhibitors, amylase inhibitors, proteases, tannins, saponins and lectins. The presence and level of these compounds in the relevant InnoProtein samples will be monitored. Nucleic acid content in the SCP samples will also be monitored.

1.3.3. Sensory and physical properties

The sensory and physical properties of amino acids in animal feed are important considerations for the formulation and acceptance of feed by animals. These properties can impact the palatability, handling, and overall effectiveness of the feed. Consideration of these sensory and physical properties is crucial when formulating animal feeds to meet the nutritional requirements of the target species and to ensure that the feed is easily consumed, processed, and stored. Producers should work with nutritionists and feed experts to optimise the formulation for their specific needs. A list of some key sensory and physical properties of amino acids in animal feed:

Appearance

The visual appearance of amino acids can vary. Amino acid powders are typically white or off-white in colour. The appearance should be uniform without any discoloration, clumps, or impurities. Visual quality is essential for quality control and ensuring the feed's appeal to animals.

Particle size

Particle size affects the mixing and flowability of amino acids in the feed. A uniform particle size is essential to prevent segregation and ensure even distribution in the feed mix. It also influences the ease of handling during feed production.

Odour and taste

Amino acids can have characteristic odours and flavours that can impact the palatability of the feed. Some amino acids may have a slightly bitter or sour taste, which can affect animal preference. Minimizing unpleasant doors or tastes is crucial to maintain animal consumption.

Solubility

The solubility of amino acids can vary, and it is important to consider this when formulating feed. Some amino acids are highly soluble in water, while others are less so. Solubility influences the ease of mixing and distribution in the feed and can affect how animals consume it.

Hygroscopicity

Amino acids can be hygroscopic, meaning they have a tendency to absorb moisture from the environment. This property can affect the flowability and storage stability of the feed, as excessive moisture can lead to clumping and reduced shelf life.

Dustiness

Some amino acid powders can generate dust when handled or mixed, which can pose respiratory and handling hazards for both animals and workers. Reducing dustiness is important for animal and human health and safety.

Bulk density

The bulk density of amino acids can impact the volume and weight of the feed. Formulators need to account for the bulk density to ensure that the correct amount of amino acids is included in the feed to meet nutritional requirements.

Mixability

Amino acids should be easily mixed with other feed ingredients to ensure uniform distribution of nutrients. Poor mixability can result in nutritional imbalances within the feed.

Flowability

Good flowability ensures that the feed ingredients can be handled, transported, and processed efficiently in the feed production facility without clogging or bridging in equipment.

Anti-caking properties

Some amino acids may be prone to caking or agglomeration, especially in humid conditions. Anti-caking agents may be added to prevent these issues and ensure that the amino acids remain free-flowing.

1.3.4. Functionalisation and purity

Functionalization and purity are essential considerations when formulating proteins for animal feed applications. The quality of proteins in animal feed directly affects the health, growth, and productivity of the animals.

Functionalization and purity in animal feed are essential to optimize animal health, growth, and product quality. Collaboration between animal producers, nutritionists, and feed manufacturers is critical to formulate feeds that meet these requirements and comply with regulations. Ongoing research and development play a crucial role in continually improving feed formulations to meet the evolving needs of the animal agriculture industry. A list of the key aspects of functionalization and purity for proteins in animal feed:

Functionalisation

- Nutritional balance: The primary function of proteins in animal feed is to provide essential amino acids required for the growth and maintenance of animals. The feed should be formulated to provide a balanced amino acid profile specific to the species and production goals.
- Digestibility: The proteins in animal feed must be highly digestible to maximize nutrient utilization and minimize waste. This often involves selecting protein sources that are easily broken down and absorbed by the animals.
- Palatability: The feed should be formulated to be palatable to the target animals, encouraging them to consume it consistently. Taste, texture, and aroma can impact feed acceptance.
- Growth promotion: Depending on the goals of animal production (e.g., meat, milk, eggs), functionalization may include additives or proteins that promote growth, such as growth hormones, enzymes, or specific amino acids.
- Immune support: In some cases, proteins may be functionalized to support the immune system of animals, helping to prevent diseases and improve overall health.

Purity

- Ingredient quality: Proteins used in animal feed must be of high quality, free from contaminants, and not adulterated with non-protein substances. This includes ensuring the quality of raw materials such as soybean meal, fishmeal, or other protein sources.
- Toxin control: Animal feed must be free from harmful toxins, such as mycotoxins, which can negatively impact animal health. Regular testing for toxin presence is essential.
- Hygiene and safety: The entire feed production process, from ingredient sourcing to manufacturing and storage, must maintain high standards of hygiene and safety to prevent contamination.
- Regulatory compliance: Feed manufacturers should adhere to relevant regulations and guidelines regarding feed quality and safety. Compliance ensures that the feed is safe for animals and does not pose health risks.
- Traceability: Establishing traceability in the supply chain helps identify the source of protein ingredients and monitor their quality. This can be crucial in addressing any quality or safety issues that may arise.

- Quality control: Regular quality control checks and testing are necessary to ensure that the protein feed meets purity standards. This may include proximate analysis, microbiological testing, and more.

1.4. Non-food bio-based applications

The relevant products to consider for non-food bio-based applications include biostimulants and plastic-free cutlery products.

Biostimulants derived and extracted from biological origins (plants, microorganisms, animals) are already available on the market and are used frequently. An example of these is EnNuVi, which is a multipurpose and versatile plant nutritional tool for farmers that is based on plant-derived polyphenols in combination with one or more essential macro and micronutrients.

Figure 1. EnNuVi biostimulants

Plastic-free cutlery products will be developed through bioplastics obtained from the denaturation of protein and chitin.

The successful development and adoption of biostimulants involve multiple stakeholders in the agricultural sector. The ultimate end-users are primarily the farmers, but the impact and utility of biostimulants extend to other key players as well, including fertilizer producers/distributors, research centres, universities, and regulatory bodies.

Products incorporating proteins in bioplastics, such as cutlery and packaging, have diverse end-users. Individual consumers and restaurants value sustainability. Catering companies and institutional food services use them to reduce waste by recovering the waste in order to incorporate it as new raw material. These bioplastic products have received interest from retail shops, which sell these products, food delivery companies that use them for packaging and also producers of bioplastics, or research centres. These products are also relevant in aviation and tourism, as well as in the vegan sector due to the use of packaging free from animal proteins. In summary, these products such as cutlery products find applications across multiple sectors as sustainable alternatives to conventional plastics.

In the formulation of agricultural biostimulants, several key attributes must be considered to ensure their effectiveness and practicality for application. These attributes include safety, stability, solubility, compatibility, absence of precipitation or coagulation, and particle size control.

During the development of formulations for bioplastics, it will be necessary to comply with the requirements of new regulations regarding safety and food suitability, while maintaining a physical rather than chemical modification in order not to modify the chemical structure of the bioplastic. Additionally, it is important to consider criteria regarding functionality, processability in common process technologies, the mechanical or thermal properties it must possess, and the use of technology and raw materials that reduce the environmental impact compared to plastic products.

For non-feed biostimulant formulation, it is crucial to know the composition of the raw materials (amino acids, peptides, fatty acids, sugars, minerals, etc.) to precisely tailor the product to the needs of specific crops or environmental conditions. For the development of bioplastics, it is also necessary to know the composition and purity of the raw materials so that the transformation process through compounding technology can be effective, and protein denaturation can be carried out.

1.5. Conclusion

The social requirements and factors are important to consider in the development of innovative protein alternatives. The protein and amino acid recommendations throughout various human age and life stages and across different animal species should be considered when designing the amino acid content of the product. The products should comply with toxicological safety standards and meet specific sensory, physical, and nutritional requirements to ensure their suitability for use in different food or feed products. Regular monitoring and testing should be carried out at different stages of production to verify the absence of contaminants and confirm purity standards.

2. Task 1.2. Market Requirements

2.1. Introduction

In the dynamic landscape of global industries, the demand for sustainable and innovative protein sources is increasing. The market dynamics of the InnoProtein proteins are examined on a global scale and on the growing European market. This analysis encompasses current market values, anticipated values over the next decade, and the influential factors who direct these markets. As the demand for alternative protein solutions gains interest all over the world, this review highlights the applications of these proteins in feeds, foods, and bioplastics, unravelling their diverse roles in shaping industries and redefining consumer choices. The regional market shares were examined and therefore providing an overview of the geographical ongoings that contribute to market dominance. But as great as the opportunities are, the challenges are just as great. Overcoming the barriers to market entry requires a thorough understanding of the hurdles that are unique but also similar to each protein source. Thus, also integrated is analyses of the barriers faced by potential market entrants and identifies strategies to overcome these challenges to pave the way for successful market introduction.

2.2. Insects

2.2.1. The global and European market of insect protein

The Global Market of insect protein is anticipated to grow from $700 million in 2022 to $6.8 billion in 2032 due to a great CAGR of 25.5% (Globe Newswire, 2023). Especially the Asia Pacific Regions are known for their long insect-consuming history and therefore, the regions with rising demand tend to be further based on other continents. The region of Europe held the largest share in the insect protein market in 2020 with more than 30%, which can be referred to a higher consumer acceptability for insect applications in food and feed opposed to other regions (Grand View Research, 2021). The European market of edible insects is estimated to reach $2.98 billion in 2030 from $271 million in 2023 by a CAGR of 29% (Meticulous Research, 2023). Within Europe, the Netherlands is forecasting to hold the largest share, since key insect manufacturers are based there and the initiatives from the government are increasing (Meticulous Research, 2023). The second largest share accounts to North America, which is anticipated to hold the largest share in 2030 due to the growing awareness of the consumers regarding the consumption of food with insect applications (Grand View Research, 2021).

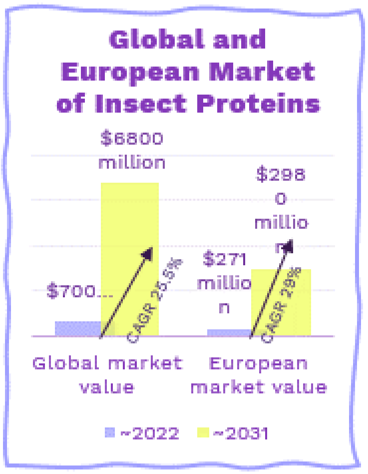


Figure 2. Global and European Market of Insect Protein

2.2.2. Segmentation

The market of insect protein can be classified into the applications from sources of, among others, mealworms, black soldier flies, buffalo worms, grasshoppers and crickets. Due to their pleasant flavour profile, high protein content and a great availability, crickets are anticipated to grow the fastest rate in the upcoming decade, at around a CAGR of 31.2% (Globe Newswire, 2023; Grand View Research, 2021). Furthermore, the black soldier fly is also expected to experience significant growth due to the expanding financial support from the government for black soldier fly as applications to livestock feed. An additional advantage that ensures the expected growth is their capability to convert waste into protein (Meticulous Research, 2023).

Whole insects are expected to account for the largest share as compared to powder, but applications in powder form are expected to grow at a higher CAGR as this is the optimal form to be incorporated into food and feed. And since the European Union has recently decided (in early 2023) that crickets and larvae insects can be used in food, a huge increase is also expected based on this new regulatory (Meticulous Research, 2023).

The different forms and extraction results from insects are able to find a broad range of application opportunities, which will be further evaluated in the following. Applications in the sectors of food, animal feed and biodegradable plastic alternatives will be addressed in more detail.

Source: Veldkamp *et al.*, 2022.

Figure 3. Insect production chain

2.2.2.1. Food application of insect protein

One of the main drivers of the market growth is the increasing demand for sustainable food products. So far, insects and insect protein already find application in protein bars, pasta, burgers and more. Even pure as whole insects there are some offers in the supermarkets (Globe Newswire, 2023). For instance, the German supermarket chain Kaufland has, since 2019, a range of different flavoured mealworms and buffalo worms in their store shelves. The founder of the main supplier of Kaufland underlines: “Customers who buy insect-based foods are primarily looking for a new eating experience. Our task is to translate this into products that fit our normal eating habits”. Furthermore, the company Veganz carried out a survey with 24,000 of their vegan constumers, which resulted in 41,6% of them, who said they would eat insects if they had been prepared decent. However, a research company did a similar survey, but on people following any type of diet, which resulted in 70% categorially rejecting insect foods, whereas 5% would try them (Schulz, 2020). Assuming these outcomes, the anyway increasing number of vegans can therefore drive the market demand for insects significantly, although they are normally strictly on plant-based foods. Furthermore, this also promotes opportunities in the meat substitute market, as the nutty/umami flavour of the insects is perfectly suitable for this application. Since the high protein content of insects, which is raging between 35% and 77%, is higher than that of pigs and chickens, insects offers also advanced possibilites in the nutrient supply of subsitute products (Schulz, 2020).

Food and beverage applications from insects currently hold the second largest share in the Global insect protein market, which tends to experience a siginifant growth over the next decade. The also increasing number of countries authorising insects in food applications, also contribute to this expected growth (Grand View Research, 2021).

2.2.2.2. Feed application of insect protein

Due to the high content of macro- and mirconutrients, insects representing a high value addition also to animal feed. Thus, it can increase the weight gaining rate of the animals, improve their digestion, is able to prevent diseases as well as vitamin deficiencies. The high quality of the contained amino acid ratio supports the muscle building in animals (Yildiz, 2022). For now, the demand for insects for feed application is mainly driven by the aquaculture market. The main driver of this market in the future of insect protein for feed application is expected to be the recent authorisation to use insect protein for poultry and pig feeding. Further authorisations will regulate which subtrates can be used for insect farming, before it will be called "waste". The mass, which would presumably called "waste", is mostly well-preserved former foodstuff, meats, catering returns, etc. Therefore not required to be called waste and perfectly usable as subtrate for insect farming. If this is an outcome, future implementation of insect protein to feed can offer at 2023 in the EU: 1% of consumed fish fed by insect protein, 1 in 40 eggs produced by insect protein fed henns, 1 in 50 chicken meat portions from insect protein fed ones and 1 in 100 serving with pig derived from pigs, which were fed with insect protein applications (ipiff, 2021; Grand View Research, 2021).

Insect protein for feed applications held the largest share in the market with more than 75% in 2020 (Grand View Research, 2021). The rather recent authorisations, which provides a high valuable and sustainable source of protein for animal feed, will be the most significant reason for an even more increasing growth in the next years.

2.2.2.3 Bio-based applications such as bioplastics

Among proteins, insects consist of further interesting ingredients for the industrial sector to produce, among other, biodegradable plastic-free plastics/packagings. As chitin and chitosan is a commonly used biopolymer for a broad range of applications in several industries, the market demand towards it is growing. However, due to the disadvantages of the up to now number-one source shellfish, such as seasonality matters wherebey availability problems comes along and ecological minimisation, the industry is looking for environmental friendlier and more sustainable alternative, providing a high availability. In point of fact, insects appearing as an optimal fitting gap-filler for the described disadvantages of shellfish. During insect farming, it occures a massive amount of sidestreams, that were previously/currently accounted as waste, such as larval/pupal remains and deceased insects, which are optimally qualified for the extraction of chitin and chitosan (Rehman, et al., 2023). Furthermore, the containing proteins and fatty acids of insects are also of great interest for this industry. For the increasing number of people intrested in sustainability alternative packaging, will be also one of the main driver in this form of insect application. However, chitin/chitosan coating in packaging entail futher advantages – to be used to provide control of physiological changes in food, control of enzymatic browning of cut fruits, increasing the shelf-life of foods, and more (straits research, 2020).

In the bioplastic market, packaging holds by far the largest share with over 60%, alongside other applications such as textiles or automotive and transportation. Furthermore, the bioplastic market is predicted to grow at a CAGR of 18.8%, increasing the market value from $11.6 billion in 2022 to $46 billion by 2030. This extremely large market will also drive the increasing demand for biodegradable plastic from insects (Grand View Research, 2022).

2.2.3. Market introduction strategies

An effective market introduction strategy, as it basically is with any new product, is essential for successful market growth. Due to the novelity of insect products, several hurdles can affect the unconventional value chain. It is essential to know and to be able to identify all conceivable hurdles in this regard, in order to be able to implement the the necessary preparations resp. implementations, alternatives or regularities for a successful market growth. Furthermore, the pricing of a protein has a siginifant influence to the market and can either present a hurdle or be an incentive to facilitate the market.

2.2.3.1. Assessment of market entry hurdles affecting the unconventional protein value chain

The journey from pilot to commercial production of insect proteins is burdened with a range of hurdles and barriers. These barriers are far-reaching, affecting every involved producer/manufacturer/seller etc. at every stage of the supply chain, from the initial farming of insects to their processing in further production to the consumers. Production barriers: At the production level, producers and manufacturers are confronted with issues such as scaling up their operations to meet commercial demands. This often requires significant investments in infrastructure and technology. In addition, insect pests and diseases can threaten the success of insect farms, requiring robust pest control measures and disease management strategies. Financial, price, and market barriers: Financial barriers coming in the form of high input costs, originating from factors such as feed and technology. Furthermore, the price of insect products is influenced by factors such as market demand and consumer willingness to pay. Societal acceptance of insect production and products represent a critical barrier that impacts not only demand but also the willingness of investors to financial support. Safety-related barriers: Ensuring the safety of insect-based products is a multifaceted challenge. Distributors often lack comprehensive knowledge regarding chemical and microbiological food safety hazards associated with insect-based products. The control measures for these hazards require continuous research and development. Regulatory barriers: Legal restrictions and the complex process of receiving licenses for insect production add another critical point in the insect industry. In addition to these hurdles, the fundamental aversion to "new" products such as insects in the consumer market is crucial for the demand (Veldkamp, *et al.*, 2022). Also, the current pricing of insect protein is rather a hurdle, due to the range of cost between 3,500€ to 5,500€ for one metric ton (Byrne, 2021). This is a significant higher price than the costs of soy protein, which costs about 650$ per metric ton (Pivotal Point Strategic Directions, 2019). If there is a lack of financial resources, for example, and not enough education can be provided, this is a major barrier to market growth. However, it's important to underline that overcoming these hurdles is essential not only for the growth of the insect industry but also for achieving the broader goals of sustainability, including reducing the dependence on traditional animal protein sources (Veldkamp, *et al.*, 2022).

Source: Veldkamp *et al.*, 2022.

Figure 4. Obstacles in the value chain

2.2.3.2. Assessment of measures and incentives to facilitate the market introduction of InnoProtein proteins

To successfully facilitate the insect protein market, it is of great importance to identify the barriers and hurdles to develop improvements and optimizations. Production optimization: one opportunity is to optimize insect farming practices is by considering the nutritional and physical requirements of various insect species. Therefore, improvements of digestibility could lead to a higher efficiency in the production process. Disease management: To address the issue of insect infections or illness, major disease agents for specific insect species should be identified, aiming to develop preventative measures. This is crucial for reducing insect mortality and maintaining the health of insect colonies, contributing to stable production. Logistics and processing: The development of a practical logistics chain for the stable storage and transportation of insects is essential for industry growth. Evaluating efficient methods and considering alternative technologies for insect processing to reduce reliance on expensive and unsustainable methods should be implemented. Innovative processing: Innovative processing techniques should be studied to create insect-based products suitable for the food, feed, and pet food industries as well as for biodegradable plastic alternative applications. Consumer engagement: for a successful market growth it is necessary to educate consumers towards the benefits of insect protein applications, to reduce the disgust against insects in foods, to underline benefits of biodegradable plastic alternatives as well as the accompanying sustainability (Veldkamp, *et al*., 2022).

2.3. Microalgae

2.3.1. The global and European market of microalgae protein

The Global market of protein from algae is estimated to grow from $750 million in 2022 to $1.2 billion by 2032 due to a CAGR of 5.5% in the next decade, whereas the specific market of microalgae protein is anticipated to grow up to a value of $820 million with the largest share in global algae protein market. Currently, the largest share holds North America (and is expected to hold it also over the forecast period) caused by the high amount of available vast aquaculture landscape and high investments of the government in aquaculture and algae-based animal feed. Futhermore, the key players in this market are mostly based in North America, which also contributes to this large share (Global

Market Insights, 2022). However, Europe is also expected to experience significant growth in the forecast period, primarily due to the increasing demand in algae supplements and natural food colourings. The European algae protein market is estimated to reach $248 million by 2026 by a CAGR of 6.5% in the upcoming decade from $160 million in 2019. The largest share in the European market is and will be held by Germany with an estimated market value of $165 million by 2028.

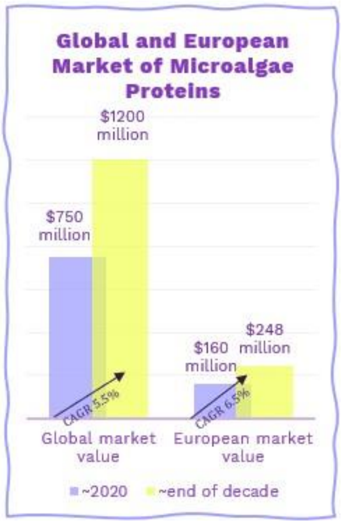


Figure 5. Global and European market of microalgae protein

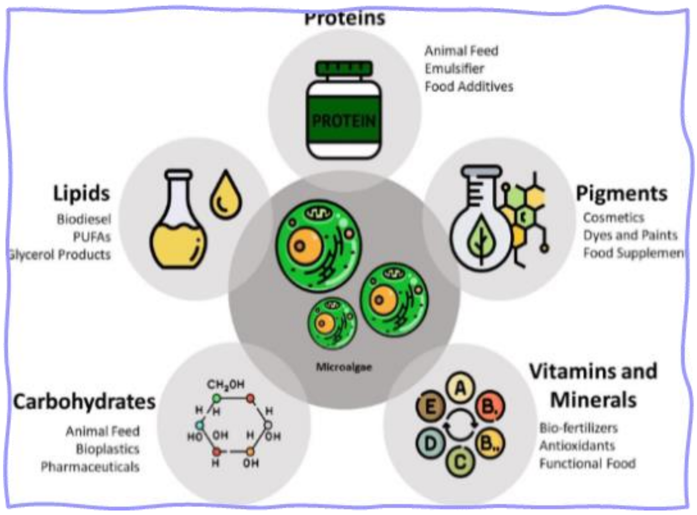
2.3.2. Market segmentation of microalgae protein

In the algae protein market, microalgae drive the market up to about 90%, opposed to macroalgae (Grand View Research, 2021). The sources of algae proteins vary between the most common ones, such as Chlorella, Spirulina, *Dunaliella salina*, *Haematococcus pluvialis* and other microalgae. In the first half of the 2020's, Spirulina accounts for the largest share in this field. This is mostly attributed to the rising demand in the area of health supporting and dietary supplements. Furthermore, the intensive colours such as bright green or bright blue, represents an interesting substitute to artificial food colourings. However, the *Haematococcus pluvialis* is expected to grow at the highest CAGR in the forecast period. In the field of extracted ingredients from microalgae, the protein segment, opposed to the ponds and fermenters segment, is expected to grow at the fastest rate in the upcoming decade. This can be attributed to the several benefits such as effect against anxiety and premenstrual heart disease or the support for weight loss and fatigue relieve. Thus, these proteins can find application in various segments, such as food &

beverages, nutraceuticals and more (Meticulous Research, kein Datum; Grand View Research, 2021).

2.3.2.1 Food application of microalgae protein

Microalgae act as a human source of nutrition since centuries. However, it was underutilized and is still today. The first species, which were introduced to the market decades ago, were Chlorella and Spirulina. These can be consumed as they are or as extracted powder applications (Acquah *et al*., 2021). The high quantity of protein in microalgae, which can be up to 70% of the dry mass, presents them as a high value source of protein. The protein-richest microalgae most commonly reported are, among other, Chlorella and Athrospira. Additionally, the amino acid profile in microalgae is well balanced and contains all essential ones, which improves their value even more. Furthermore, the recommendations from the FAO and WHO for the amino-acid balance in human consumption are perfectly covering the protein profile of Athrospira (Lucakova *et al*., 2022). Therefore, an addition of protein extracted from microalgae present a valuable source of this macronutrient. The foam stability of microalgae is another characteristic of high value. Thus, this characteristic is appropriate for the production of e.g., protein bars, where conventionally milk proteins are used, due to their foaming stability. Another helpful characteristic of protein from microalgae are their great water-holding capacity, which can support reducing moisture loss as well as the maintenance of freshness in foods - especially in bakery products - while also improving their mouthfeel. Fat absorption as another functional property in proteins, especially for flavour promoting effects, which can be found to be in Athrospira higher than in soy protein (Acquah *et al*., 2021). Today, beside powders, capsules or tablets, there are also some noodles, bread, or pasta with microalgae applications on the market (Lucakova *et al*., 2022).



Source: Machado *et al*., 2022.

Figure 6. Ingredients extractable from microalgae with applications options

The algae proteins market is dominated by the application in food and beverages as well as usage as dietary supplements – and still will be in the upcoming decade. Especially for people with interest in the vegan diet (which number is also steadily increasing), algae applications to the human diet will not only be valuable source of protein but also comes along with beneficial amounts of vitamins and minerals (Global Market Insights, 2022).

2.3.2.2 Feed application of microalgae protein

Since the 1950's, the application of microalgae to animal feed is common pratice to improve its nutritional value due to the high micro- and macronutrient density, especially the high protein content. At the moment, about 30% of the globally commercially produced microalgae are used for feed applications. Therefore, this application can also improve animals physiological health as well as their meat quality. Various experiments with microalgae in feed showing further improvements e.g. increased weight gain in lambs and broilers or an increased content of omega-3 fatty acids in silver fish. Also, in the recent years the price of fishmeal increased, which led manufacturers searching for cheaper alternatives, where microalgae went more and more into the focus. Other applicable low-cost ingredients in fish feed, such as grains and oilseed can cause siginificant changes in the quality of the final fish produced, due to big difference from natural animal feed. Microalgae is very similar to natural fish feed in that it is a much more suitable alternative due to its ecually low cost and due to its already mentioned very balanced amino acid profile (Guedes *et al*., 2015).

The application of microalgae protein to animal feed holds the largest share, directly after food & beverages and dietary supplements. The share is anticipated to double in the forecast period (Grand View Research, 2021).

2.3.2.3 Bio-based application such as bioplastic

The increasing quantity of fossil-based plastic waste is becoming more and more a problem, due to its characteristic to not biodegrade. The alternatives currently on the market holds a share of barely 1%. This small share is lead by starch blends, e.g. from corn or potatoes, which cultivation need lots of water, land and nutrients. However, on the basis of the increasing interest in sustainability, the market is expected to grow enormously in the following years. Therefore, microalgae can be a more appriopiate alternative to starch blends, due to their ability to subsist off "waste" resources. The most common species for this form of application are Chlorella and Spirulina, which can be used for the production of biopolmers as well as plastic blends. Many ingredients of bio-based products can be used for plastic-free "plastic" production such as proteins or carbohydrates. However, due to the moreover complex extraction/production processes needed, the industrial upscaling is the major problem in further commerzialisation of microalgae-based plastic alternatives. Thus, further research and process optimizations need to be implemented in the future (Cinar *et al*., 2020).

2.3.3. Market introduction strategies

Within the present state of market development, there are significant challenges and hurdles associated with the utilization of products containing microalgae protein. These hurdles are mostly based in the beginning of the value chain, which must be overcome, for example with improved technology.

2.3.3.1. Assessment of market entry hurdles affeecting the unconventional microalgae value chain

Several significant challenges and barriers impede the market introduction resp. a sucessful market growth of microalgae products. These include, among others, the following challenges. High production costs: the production costs associated with microalgae biomass and its extractable ingredients are high. Recent estimates suggest a cost of 3.4 EUR/kg for dry weight microalgal production in Spain and 5.1 EUR/kg dry weight for *Tetraselmis suecica* production in Italy. Lack of understanding: There is a lack of knowledge concerning the effects of consuming microalgae biomass, its digestibility, and overall safety. This knowledge gap presents a critical challenge in the successful incorporation of these components into various products. Insufficient research: Incomplete research efforts have been directed towards the development of new food products that include microalgae components. This deficiency in research represents a significant obstacle to further progress in this field. High protein separation costs: The extraction of functional proteins from microalgae requires gentle processing conditions, resulting in currently enormous costs that hinder economic viability. While novel techniques like pulse electric field (PEF) and ultrasound have the potential to reduce energy consumption for cell disruption by up to 90%, they face limitations, such as limited availability and high initial setup costs. Legislation procedures: Every algae species, which has not been included into food products before the 15th may 1997, has to undergo the Novel Food authorization procedure to ensure their safety for human consumption (Lucakova *et al*., 2022). High overall costs/product costs: For example, Spriulina and Chlorella, which is mostly selled as a whole product compound, cocts about 2,400€ to 6,600€ per metric ton (Eilam *et al*., 2023). Addressing these challenges is essential to unlock the full potential of microalgae-derived bioactive components in a wide range of applications.

2.3.3.2. Assessment of meausres and incentives to facilitate the market introduction of InnoProtein microalgae proteins

Measures and incentives to facilitate the market introduction of microalgae products are crucial for their successful integration into the various industries. To address the process of ensuring the safety of microalgae, it is essential to focus on the strict safety protocols and research. Potential hazards associated with microalgae consumption include heavy metal poisoning, mycotoxins, pesticide residues, and pathogens. Furthermore, the presence of anti-nutritional factors and allergens, along with substance modifications during processing that may increase allergenicity, must be examined. In addition to safety concerns, the digestibility of microalgae proteins requires further exploration. While some studies report high digestibility rates, others suggest lower values compared to traditional protein sources like eggs, soy, and peas. The development of standardized methods for assessing digestibility and bioavailability is essential. To promote the market introduction of microalgae, improving processing methods is of highest importance. Techniques such as cell disruption methods and molecular weight cut-off filtration can enhance nutrient absorption and generate user-friendly powder formats. Research and development funding policies should prioritize these advancements, particularly in Europe. Although challenges exist, the future for microalgae appears promising. They offer valuable sources of, among others, proteins, aligning with global goals like climate neutrality, biodiversity protection, circular economy development, and sustainable food systems. The European Commission's Green Deal, with its focus on these areas, can serve as a significant driver for microalgae development in Europe and beyond (Lucakova *et al*., 2022).

2.4. Fungi

2.4.1. The global and European market of fungal protein

The global market of fungal protein was rated at $156.6 million in 2020 and due to a CAGR of 9.5%, it is estimated to grow up to $386.6 million in 2030 (Allied Market Research, 2021). North America is currently holding the largest share of the global fungal protein market due to the rising preference for high-quality ingredients of their consumers as well as th good availability in this region. The growing vegan culture also contributes to this market share. The region of Asia-Pacific is estimated to grow at the hightest CAGR in the following decade which can be accounted to the also rising health awareness among the consumers and the growing demand of vegan food alternatives. Additionally, the production of fungal protein in India and China are growing which also attributes to the expected high CAGR (Meticulous Research, 2023). Europe held a share of $57.8 million in 2020 (Allied Market Research, 2021) is is anticipated to grow up to $103.8 million in 2030 due to a CAGR of 5% and can be attibuted to the gaining interest in antioxidantal and disease-preveting properties of fungal protein in the pharmaceutical field. These required properties can be related to the rising elderly population, which increased the number of people suffering chronic diseases (Global Market Insights, 2022).

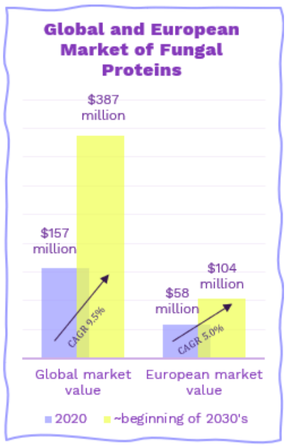


Figure 7. Global and European Market of fungi protein

2.4.2. Market segmentation of fungal protein

The fungal protein market is categorized by type. Within this classification, yeast snacks emerge as a dominant segment, holding a great share in the market in 2020 and projected to maintain its dominance throughout the next decade. Another segmentation criteria is based on the nature of the products, differentiating between organic and conventional fungal protein. This segmentation is particularly relevant as consumers increasingly show a preference for organic and environmentally friendly products. Thus, opportunities for market growth are created. Additionally, the market is segmented by application, such as food and beverages, animal nutrition, pharmaceuticals, and other sectors. However, the increase in consumer willingness to invest in premium and environmentally friendly products represent a promising way for the future growth of the fungal protein market (Meticulous Research, 2023).

2.4.2.1. Food application of fungal protein

Fungal protein as food application is also well researched an implemented. As food application, it is mostly used as a meat substitute in forms of burgers, strips or cutlets. Already in 1983, it was approved as safe by the U.K. Ministry of Agricultre, Fisheries and Food, whereas in the U.S. it was first registered as safe in 2001 (Frothingham, 2019). A well established brand which produces fungi-based meat substitues from *Fusarium Venenatum* is Quorn, founded in the 1960's in the U.K. due to the protein crisis in the 60's. Nowadays, Quorn sells its products all over the world and encounters great interest among vegans and vegetarians looking for meat substitutes. As it can be seen in Figure 7, Quorn-products showing less environmental impact in form of CO_2-emissions than comparable products (Quorn, 2020). Fungal protein is also known to have blood cholesterol lowering characteristics, especially in humans who are suffering high cholosterol quantities. Furthermore, opposed to animal-based protein sources such as chicken, fungal protein shows advantages regarding satiety, which can help to lower energy intake and can support weight loss (Filho *et al*., 2019).

Source: Quorn, 2020.

Figure 8. Environmental impact of Quorn opposed to alternatives

2.4.2.2. Feed application of fungal protein

Applications of fungal protein in feed are also well researched. In 2019, Filho *et al.* examined the opportunity to grow fungal protein on waste water as sidestream from starch production, where almost double the amount of waste water per part wheat occures. Where normally microbial removal procedures would begin, the researchers used it to cultivate the fungi biomass on it. Therefore, almost zero greenhouse gas emissons were procuded during the cultivation, which shows a very sustainable protein production. The resulted fungi present a high value protein source as feed additve.

PEKILO®, developed by a Finnish biotech start-up, is another successful fungus-based feed application. It is grown on diverse by-products such as from the forest-, agri- or food production. It contains up to 70% protein and, beside high quality fibres, a high concentration of vitamin b. The process of production needs comperatively small amounts of land and water. For example, PEKILO® is used in aqua feed as a soy alternative and shows also improvements in the growth performance and immune response in fish (Fish Farmer Magazine, 2023).

2.4.2.3. Bio-based application such as bioplastic

Currently, there are very limited informations available using extracted protein from fungus as bio plastic application while more references through using the mushroom as a whole could be found. For example, the EU-project FUNGUSCHAIN used mushroom residues for the production of biodegradable plastics. The cultivation of mushrooms for human consumption generates about 25% of waste, which would normally not be used futher, but within the project, this redisues were taken for the conversion to fungus-based bio-plastic application (Tecnaro, 2021).

2.4.3. Market introduction strategies

Introducing fungal proteins to the market demands a strategic approach that emphasizes their nutritional advantages and aligns with consumer preferences. Recognized as a sustainable protein source with a minimal ecological impact, fungal proteins offer a promising alternative. As the demand for sustainable and innovative food sources grows, implementing effective market introduction strategies becomes paramount for the widespread acceptance of fungal proteins. The price of the final mycoprotein product is estimated to $21.80/kg which corresponds to, depending on the final protein content, around $173 per kg pure protein (Upcraft *et al*., 2021).

2.4.3.1. Assessment of market entry hurdles affecting the unconventional fungal protein value chain

Several hurdles and challenges are affecting the value chain of fungal protein. Technological challenges: in the past, the dominance of Quorn in the market of fungal protein applications has resulted in limited competition and innovation. The patent which was held by Quorn is expired and therefore opened the market to new players. But high capital costs and the risk of patent infringement as well as limited availability of resp. species did not make the market entry any easier for new players. Regulatory hurdles: Inconsistencies in labeling, such as the 'mushroom-based' designation in the US without reference to mold, have led to legal action and increased regulatory inspections. The need for transparent labeling and adherence to allergen disclosure requirements adds complexity to market entry, particularly in regions like the US. Consumer perception hurdles: Despite its nutritional benefits and environmental sustainability, fungal protein remains relatively unknown among consumers. The general lack of awareness, compounded by misconceptions (e.g. with mold), hinders the widespread acceptance of fungal protein-based products. Further, categorization confusion is a problem. The unique nature of fungal protein, distinct from both plants and conventional meat, often results in misclassification. Consumer confusion, compounded by the use of terms like 'vegan' but not 'plant-based,' may impact the positive attitudes typically associated with plant-based foods. The meat attachment and neophobia is a problem which has to be overcome through information. Consumer attachment to traditional meat, influenced by personal, social, and cultural values, poses a challenge to the adoption of fungal protein. Food neophobia, or the unwillingness to consume novel foods, further complicates consumer acceptance. Strategies to eliminate these concerns, such as transparent labeling and reduction in price relative to meat, become crucial for market success. Market positioning: Fungal protein, despite being available for over three decades, is currently perceived as a premium and specialist product. Shifting this perception and promoting wider adoption may require strategic pricing relative to traditional meat products (Chezan *et al*., 2022).

2.4.3.2. Assessment of measures and incentives to facilitate the market introduction of InnoProtein fungal proteins

The systematic review (Siddiqui *et al*., 2022) emphasises the diverse drivers influencing consumer acceptance, underlining crucial considerations for the successful market introduction of fungal protein. To navigate the complexities associated with consumer choices, a multifaceted approach is essential. Firstly, comprehensive marketing strategies should be implemented to educate consumers about the benefits and advantages underlying the consumption of fungal protein. Highlighting the sustainability and nutritional advantages of fungal protein, along with addressing any existing misconceptions, can play a important role. Secondly, there is a need for intervention studies focusing on information dissemination and enhancing consumer education with fungal protein. These studies should underscore the unique environmental benefits of fungal protein, positioning it as an eco-friendly alternative. Additionally, the systemic review underscores the importance of legislative frameworks tailored specifically to alternative proteins. Aligning demand with market supply necessitates a strategic approach, emphasizing the development of standardized measures for consumer acceptance. Future research initiatives should prioritize comparative studies across different alternative proteins, including fungal protein, using standardized measures that account for personal, social, and physical variables. By implementing these measures and incentives, the market introduction of fungal protein can be facilitated, fostering consumer acceptance and driving its integration into mainstream dietary choices.

2.5. Bacterial protein

2.5.1. The global and European market of bacterial protein

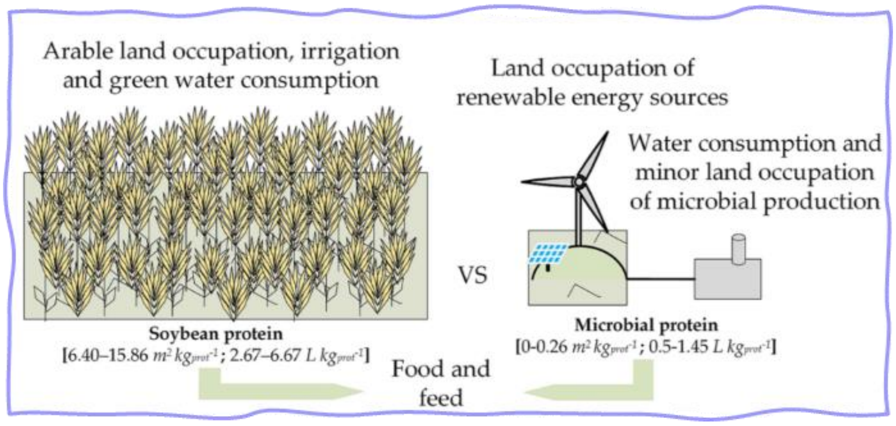
At the moment, the market for bacteral proteins is still quite underexplored. Market reports are mostly summarizing the whole market of microbial proteins, which includes algae, yeast, fungi and bacterial. Thus, no clear data in numbers for only bacterial proteins are available. However, proteins from bacteria are expected a growing at a CAGR of 7.9% (SKYQUEST, 2023). In the past, the growth of the market of bacterial proteins more ore less stopped due the high productions costs opposed to cheaper e.g. soybean proteins. However, according to the growing interest in sustainability and reducing land and water use, bacterial proteins are acquiring once again more focus, especially in the feed industry. The largest share in the market of microbial proteins is held by North America, where the interest is growing due to environmental concerns and ethical aspects. During the next decade, the region of Asia-Pacific is forecasted to grow at the highest CAGR (Matassa *et al*., 2016).

2.5.2. Market segmentation of bacterial protein

The fact, that bacterial protein ca be produced from the nutriens and organic carbon from sidestreams such as "waste" or processing water, is a high sustainable and enviromentally conscious option. Moreover, it should be feasible to manufacture this bacterial protein at a cost that considers the revenue generated from preventing the treatment of mineral nutrients found in side streams (waste). Although the tests in fish feed were sucessful, the manufacturers had no breakthrough due to the low prices of soy protein in the 1970's (Matassa *et al*., 2016).

2.5.2.1. Food application of bacterial protein

In about 2019, scientists from Finland developed a process to produce protein "from thin air" which means, it is produced by soil bacteria fed by hydrogen split from water by electricity. Furthermore, if the electricity would come from wind or solar power, these proteins can be produced with nearly to zero greenhouse gas emissions, which would be the most sustainable possible way to produce food, opposed to e.g. soy bean cultivation. Despite the fact that uscaling will require several more years, it already had a very neutral flavour in the development phase, which, in addition to the almost non-existent greenhouse gas emissions, makes it a really important alternative for the future of human nutrition. Additionally, these protein can also be used for growing cultured fish or meat (Harrabin, 2020).



Source: Sillmann *et al*., 2019.

Figure 9. Land-use soybean opposed to bacterial protein production

2.5.2.2. Feed application of bacterial protein

Until today, bacterial protein is mainly used in animal feed. Bacterial protein could be a valuable alternative against the use of plant-based protein with applications in feed as well as for other applications such as food for human nutrition. However, some simulations show that bacterial protein can just used as exchange for other proteins in small quantities due to their lack of balanced amino acid profiles; it can at least save a certain amount of land-use to produce other proteins and can present a footstep further to a more sustainable system.

2.5.2.3. Bio-based application such as bioplastic

About 90 types of bacteria, including archaebcteria, photosythetic ones, aerobes, anaerobes and lower eukaryotes, are able to produce certain types of polymers, which could potentially be further processed to bioplastic material. However, the upscaling of these processes are limited due to high costs which cannot compete with alternatives. So, to date there are around 160 different polyesters produced by microorganisms (not just bacteria!). It can be said that further research in this field, with a special focus on the diversity of possible microorganisms, could create further types of bioplatics (Luengo *et al*., 2003)

2.5.2. Market introduction strategies

As the use of bacterial protein is not very widespread, market is in a very incepious stage. So far, however, the characteristics of bacterial protein, such as almost zero greenhouse gas emissions or the potential land use savings, could be promising arguments for a successful market entry.

2.5.3.1. Assessment of market entry hurdles affecting the unconventional bacterial protein value chain

As the production and processing of bacterial protein is not yet very widespread, a great amount of research and investment is required to ensure a successful market entry. The following text addresses the hurdles and barriers that need to be overcome.

Production barriers: During the production of bacterial protein, the energy-to-biomass efficiency determines the production costs, which can vary massive. High required amounts of energy at this time of very high energy prices is a factor which causes a lower competitiveness to other sustainable protein alternatives. Therefore, it is important to improve the effiecency for the production of bacterial protein. Furthermore, the use of wastewater requires strict safety processes, which weighs also in terms of costs. Safety and regulatory hurdles: Downstream processes present challenges related to pathogenicity, toxic compounds, and the need for a sterile production environment. Approval for human consumption demands rigorous nutritional and toxicological testing, incurring substantial time and financial investments. Consumer acceptance hurdles: Most consumers have never come into contact with bacterial protein and therefore have no information about the benefits and possibilities (Sillmann *et al*., 2019). On the basis of the production, which has been researched very rarely so far, especially with regard to upscaling, it is not yet possible to make a price per kg estimation. As it can be seen in the previous argumention, a lot of research and investment has to be done to overcome these hurdles.

2.5.3.2. Assessment of measures and incentives to facilitate the market introduction of InnoProtein microbila proteins

To ensure a successful market introduction, it is important to take a detailed overview of the hurdles and barriers and develop improvement or remedial strategies on this basis.

Production optimization: Although the use of waste water comes with a lot of requirements regarding safety processes, it also comes with a lot of sustainable avandtages, such as no land-use and the immunity to climate changes. Assesment, whether the advantages outweigh the disadvantages or whether the disadvantages can be overcame has to be considered case by case. Furthermore, the location-independent production can be a great advantage for places with limited to no arable land. The energy-intensive nature of bacterial protein production, particularly the significant energy demand from electrolysis, necessitates a thorough assessment of energy efficiency and associated costs. Techno-economic research becomes crucial to align the technology with current market dynamics and future developments. Market uses : While the primary use of bacterial protein has been as animal feed, its potential as a human food source is gaining attention. Incentives should be directed towards exploring sustainable food systems, considering bacterial protein as a valuable resource that can save plant-based proteins for human consumption or other essential purposes (Sillmann *et al.*, 2019).

2.6. Conclusion

The market of insect protein has now and in the following decade by far the highest market value, both globally and in the European region, opposed to the three other protein sources. Both markets will grow by far at the highest CAGR, almost 6 times higher than that of the algae and fungal protein market. The markets of insect and fungal proteins in Europe are anticipated to grow at a higher CAGR than globally, whereas the algae protein market is estimated to grow faster globally. This could be due to the fact that the key players in the insect and fungal protein markets and research in this area are based more in Europe, while the algae protein market is more localised in America, where there is also a lot of financial support from the government.. The main growth driver of all applications is definitely the increasing demand for more sustainable products in every respect, whether it's food, feed or bio plastic applications. Based on this research, the kg prices of insects and microalgae protein are in the same range, between \$2.4 and \$6.6, while fungal protein is higher, at just under \$22/kg. It is still unclear where the price of microbial protein will stabilise. However, what they all have in common is that they differ significantly in price from the cheap soya protein which is around $0.65/kg. With regard to food applications, all proteins have already found widespread use, insects for example in burgers and pasta, microalgae protein more in the food supplement sector but also in protein bars. Fungal protein has so far been used by the market leader Quorn as the main ingredient in its products and is therefore successful worldwide. In the feed sector, all proteins have also already found application, in the case of microalgae, microbial and insect protein, it has so far accounted for the largest share of the market in each case. Bio plastic applications have so far been used less in all areas (exept fungal protein, where currently no information of application could be found), but research in this field is increasing. In the insect area, sidestreams are used for extracting chitin and chitosan to produce biodegradable plastics. When it comes to barriers of market entry, all four sources share the first common problem: high production costs. Upscaling is always a challenge and requires high and therefore risky investments. In the case of the fungal protein, the market leader had a patent on its product for a long time and was successful with upscaling. What also all alternative proteins analised within this report have in common, is the lack of consumer education with regard to benefits and clarification of misunderstandings (e.g. in the case of fungal protein, which many people associate with mold due to a lack of knowledge). Another common challenge represents the legislation related to novel foods, which entails long processes of investigations and approvals, which in turn can cause investors to feel uncomfortable to invest. In general, it can be concluded that all areas, especially in relation to upscaling, still need further research and consumers need to be better educated/ informed in order to be convinced of the advantages. Also, additionally to the sustainable and environmentally friendly aspects the sensorial aspects (especially taste) of most alternative proteins products have to be considered/optimized for a better acceptance by the consumer and consecutively a succseful market.

3. Task 1.3. Environmental Requirements

3.1. Introduction

In the face of growing global challenges such as population growth, climate change and resource depletion, the need to develop sustainable solutions is more pressing than ever. A key area in this quest is the creation of novel proteins that not only meet the growing demand for nutrition but also meet stringent environmental criteria. The urgency to innovate in protein development stems from the unsustainable practices associated with traditional livestock farming, which not only contribute significantly to greenhouse gas emissions, but also require high land and water resources. As environmental concerns grow, sustainable protein development becomes paramount, requiring research, ethical considerations, and a commitment to mitigate our ecological footprint. Thus, environmentally friendly protein synthesis emerges as a promising solution for a transformative shift towards a more resilient and ecologically responsible food system.

For this reason, the objective of Task 1.3 is to compile an inventory of the environmental requirements that will have a direct or indirect impact on InnoProtein production. This inventory will serve as a reference guide for conducting environmental assessments related to InnoProtein, offering valuable insights into the sustainability, feasibility, and scalability of the novel protein production processes on an industrial scale.

3.2. Materials and Methods

The analytical methodology employed in achieving this study involved a comprehensive review of the existing scientific literature and state of the art resources. This encompassed life cycle assessments and sustainability metrics, with a specific focus on single-cell protein production derived from microalgae, bacteria, fungi, and multi-insect system production. The information found will serve to define the environmental prerequisites and potential risk factors to consider in the development on the different InnoProtein processes. The review was done based on the following classification:

1. *Biomass production:*
    a) *Microalgae*
    b) *Bacteria and fungi*
    c) *Multi-insect system*
2. *Protein recovery:*
    a) *TPP*
    b) *UAE*
    c) *ASE and NADES*
3. *Valorisation of residual biomass:*
    a) *Chitin – chitosan*
    b) *SCP fermentation medium*
    c) *Bioplastics and biostimulants*

3.3. Results

3.3.1. Biomass production

3.3.1.1. Microalgae

Over the past few years, microalgae have emerged as significant agricultural resources for the global food and beverage industries, aqua-farming, and both animal and human nutrition. This trend can be attributed to several key factors (Wang *et al*., 2021):

- Exceptional Nutrient Profile: Microalgae are rich in protein, essential amino acids, and an array of healthful components, including vitamins, antioxidants, omega-3 polyunsaturated fatty acids, and essential minerals.
- Sustainable Cultivation: Microalgae stand out for their long-term sustainability as they leave the smallest carbon, water, and arable land footprints among all crop options.
- Environmental Benefits: Microalgae contribute to environmental pollution remediation, providing ecological services that aid in restoring and improving ecological balance.
- Enhanced Productivity: Compared to traditional terrestrial crops and animal-based foods, microalgae demonstrate significantly higher levels of productivity.

While the potential advantages of microalgae are clear, only a limited number of species have made it to the market (mention species). To growth the market and attract investments, uncertainties in microalgae-based processes and products need to be addressed. Successful technology should not only meet techno-economic requirements but also undergo rigorous environmental assessments to gauge its commercial viability.

Furthermore, ongoing efforts are required to optimize processes and enhance the efficiency of microalgae-based products, especially considering the substantial downstream processing costs. Determining optimal parameters is essential for scaling up and integrating upstream and downstream steps efficiently. Developing sustainable and cost-effective microalgae technologies could confirm their prospective benefits and support future research and development investments.

Additionally, given the substantial energy demands of microalgae processes, the need for clean, sustainable, economically effective, and efficient energy production systems remains a significant global challenge. To align strategic and scientific initiatives effectively, government incentives and industrial managers should conduct detailed environmental analyses of renewable energy matrices. This will help identify demands and bridge research gaps, facilitating a transition toward a sustainable biotechnological industrial future (Deprá *et al*., 2020).

Environmental aspects

Various life cycle assessments from the literature regarding microalgae production reveal that the most significant impacts are observed in categories such as non-renewable energy consumption, global warming, and respiratory inorganic emissions. These impacts are largely associated with microalgae cultivation in specific scenarios and cleaning procedures in others. The viability of microalgae as a protein biomass source is limited due to the low concentration of microalgae in large volumes of water, which requires constant dynamic maintenance and temperature control to adapt to changing environmental conditions.

The energy-intensive nature of microalgae-based processes is primarily driven by cultivation, dewatering, and drying operations. Harvesting alone can account for a significant portion (approximately 20-30%) of the total cultivation cost, while downstream processes may contribute up to 60% of the costs. Downstream processing technologies are recognized as major contributors to environmental impacts, posing a substantial challenge in the development of commercial products (Deprá *et al*., 2020).

For that reason, it is important for selecting efficient dewatering and drying methods. Specifically, centrifugation and filtration are highlighted as effective dewatering methods, while freeze-drying and spray-drying are identified as suitable drying methods due to their applicability and their ability to preserve the integrity of intracellular compounds (Deprá *et al*., 2020).

The critical point identified in the harvesting phase is linked to centrifugation, an energy-intensive equipment used to obtain concentrated microalgae sludge. Centrifugation's efficiency is highly dependent on various factors, including the characteristics of microalgae species (such as size, morphology, and specific gravity), cell sedimentation, sludge retention time in the centrifuge, and sedimentation depth (Deprá *et al*., 2020).

In open raceway pond systems for food and feed purposes, other factor of high energy consumption arises from the challenge of maintaining consistent temperature conditions throughout the year. This involves heating the systems during winter, cooling them in summer, and ensuring fluid mixing within the systems. These findings raise significant environmental concerns, emphasising the need to optimise climate conditions for systems that rely on substantial water volumes for production. Interestingly, despite existing literature often suggesting high energy requirements for microalgae processing, some assessments showed that the actual processing of microalgae biomass was found to contribute only 5-10% to the overall environmental impact (Smetana *et al*., 2017).

Strategies to improve environmental impact

Various strategies for reducing energy requirements in microalgae processing are found in the literature.

First, the use of pressurized filtration and its combination with spray drying is evaluated (Deprá *et al*., 2020).

- Pressurized filtration is more energy-efficient (0.08 kWh/m^3) compared to conventional centrifugation methods (up to 8 kWh/m^3), but centrifugation methods are still commonly used due to their adjustability and higher centrifugal working capacities.
- Centrifugation and freeze-drying methods were considered for biomass drying, but they substantially increased energy demands, particularly in raceway ponds.
- The viability of the freeze-drying method is limited to industrial processes with lower workloads and specific requirements for wet biomass.
- The combined method of pressurized filtration and lyophilization is evaluated, resulting in lower energy requirements for drying *Chlorella vulgaris* and *Arthrospira* platensis, below 20% of the total required energy.
- While this combination represents the best method in terms of energy requirements, technological improvements are needed due to the low workloads,

instability, and slow processes associated with freeze-drying and pressurized filtration. Maintenance of auxiliary equipment and sterilization of freeze-dryer chambers also contribute to increased investment costs.

- Regarding upstream stages, energy demands are influenced by the specific morphological characteristics of each microalgae species. Currently, commercially successful microalgae species thrive in selective environments, overcoming contaminating microorganisms in open systems.

Second, the implementation of other energy and carbon sources.

Combining microalgae bioreactors with photovoltaic panels emerges as a promising alternative to enhance overall efficiency and minimize environmental impacts. This configuration improves photoconversion efficiency, provides electrical energy alongside microalgae biomass, and helps mitigate photoinhibition phenomena. Transitioning to clean energy sources like wind, hydroelectric, and photovoltaic power holds the potential for substantial decreases in environmental impacts, potentially reducing greenhouse gas emissions by up to 95% (Smetana *et al*., 2017).

Shifting energy sources in heterotrophic microalgae production has the potential to decrease environmental impact by 40%, while modifying the carbon source could lead to a reduction of 25-40%. The most favourable scenario entails utilizing food waste as a carbon source and adopting photovoltaic energy generation for cultivation, resulting in a protein concentrate powder with a significantly reduced integrated environmental impact. For example, using alternative carbon sources, such as glycerol, and different microalgae strains (e.g., C. pyrenoidosa fed with food waste) had the potential to improve microalgae production with reduced environmental impact (Smetana *et al*., 2017).

Cultivating in more favourable climate conditions decreased environmental impact by reducing the need for heating but led to higher water consumption due to increased evaporation. Nonetheless, open raceway pond scenarios maintained a relatively high environmental impact compared to other scenarios (Smetana *et al*., 2017).

Optimizing carbon and energy sources, as well as climate conditions, can significantly reduce the environmental impact and promote more sustainable alternative protein production.

Finally, Table 9 summarises the most affected environmental impact categories in both cultivation methods (Smetana *et al*., 2017):

Table 9. Environmental requirements for cultivation methods of microalgae biomass

	Autotrophic Cultivation	Heterotrophic Cultivation
Energy Consumption	Autotrophic microalgae cultivation relies on photosynthesis, which is energy-efficient since it uses sunlight as the primary energy source. However, the construction and maintenance of photobioreactors and other infrastructure may have energy and resource impacts.	Heterotrophic cultivation typically relies on external carbon sources, which can be associated with higher energy consumption, especially if the carbon sources are energy-intensive, such as glucose.
Resource use	This method generally has lower resource requirements, as it primarily depends on sunlight and nutrients. However, water usage can be a concern in arid regions.	The use of external carbon sources can lead to resource consumption, including water and raw materials to produce carbon substrates.
Carbon Emissions	This method tends to have lower carbon emissions due to the use of sunlight for energy. However, emissions may occur in the production of photobioreactors and associated infrastructure.	The carbon emissions associated with heterotrophic cultivation are often higher, especially if the carbon sources come from fossil fuels or energy-intensive processes.
Waste Generation	This method usually generates less waste, as it relies on natural processes like photosynthesis.	The disposal of waste from carbon substrates and other inputs can be a concern in heterotrophic cultivation.
Chemical Usage	Relatively lower use of chemicals, as natural nutrient sources can be utilized, but the control of nutrient levels is important to prevent eutrophication.	May involve higher use of chemicals for nutrient supplementation and pH control, which can have environmental consequences.

3.3.1.2. Multicellular filamentous fungi

The Fungi Kingdom comprises heterotrophic eukaryotes whose forms vary from microscopic, single-celled yeasts to macroscopic multicellular mushrooms. Some common characteristics of the Kingdom include the cell wall formed of β-glucan and chitin, the presence of the aminoadipidic pathway for the biosynthesis of lysine, and the presence of flattened mitochondrial cristae. In nature, fungi play an essential role in nutrient recycling and support plant life in the form of endophytes and mycorrhizae, providing nitrogen, phosphorus, and other elements in exchange for photosynthesis-derived metabolites. According to their life cycle, fungi are divided into three major groups, namely, unicellular (yeasts), macro filamentous (produce fruiting bodies known as mushrooms), and multicellular filamentous. InnoProtein is particularly focused on multicellular filamentous fungi to produce Single Cell Proteins (SCP).

Multicellular filamentous fungi are characterized by their growth as septate hyphae, i.e., branching filaments that are divided into distinct sections, which get entangled to form the mycelium. Those filamentous fungi belonging to Zygomycota and Ascomycota phyla are the most interesting ones for industry, due to the broad range of possible substrates that can be used, and the end products produced. Zygomycota is divided into two classes: Trichomycetes and Zygomycetes, which are found worldwide as saprophytes growing on dead organic matter. Zygomycetes are closely related to food applications, the classical example being Rhizopus oligosporus, which is used for tempeh preparation. The main advantages related to Zygomycota are their high metabolic plasticity and high growth rate, which make them able to grow in a wide range of environments and produce a high variety of hydrolytic enzymes.

The technology used in the production of protein depends on the fungal morphology. While yeasts are preferably grown using the submerged fermentation technology, moulds and mushrooms can be grown in solid-state fermentation (SSF) systems, as the metabolism of filamentous fungi is less affected by the moisture content of the medium for a broader range of values. The production of mushrooms is carried out in solid-state, and the substrate used depends on the type of mushroom (e.g. sawdust, lignocellulosic materials, composts) (Souza Filho, 2022).

Solid state fermentation

Solid-state fermentation (SSF) is a bioprocess where microorganisms grow on moist solid substrates, using these substrates both as inert carriers and as a source of carbon and nutrients. In SSF processes, enough moisture is present to support microbial metabolism, but the free water content is very low and, therefore, the air is in the continuous phase. Filamentous fungi have some unique morphological characteristics that make them an excellent agent for SSF. The tubular hyphae, which emerge from the spore, elongate, and lead to the formation of new branches that can colonize and penetrate the solid substrates. This process produces a porous three-dimensional structure that is known as mycelium and facilitates the access to nutrients and air. Microscale phenomena of fungi growth on a solid substrate can be described in several steps according to the gas, water, nutrients, and enzyme exchange. Generally, most of the oxygen used by the fungi to grow comes from the gaseous phase. This oxygen passes through the solid particles by diffusion while fungi grow and releases hydrolytic enzymes. These enzymes diffuse across the substrate and help to degrade complex polymers releasing soluble nutrients. The soluble nutrients are now available to be metabolized by the fungus, which will need to absorb more oxygen and release it as carbon dioxide. The respiration process also releases water, which can be transferred to the gas phase or be absorbed by the substrate and be used by the fungi. While fungi grow, hypha elongation occurs, and metabolites are released.

SSF presents several advantages in comparison to submerged fermentation (SmF) technology. However, operational conditions and process control, are some aspects of SSF that still need to be solved to allow a successful scale-up of this technology. Some of the problems that hinder the industrial implementation of SSF are overheating, aeration and agitation optimization, or moisture and effective temperature control, as well as the assurance of an aseptic or hygienic environment. These aspects hinder the design of scalable bioreactors and the development of mathematical models that could successfully address material heterogeneity and improve heat transference and control. From the microbiological point of view, filamentous fungi growth is also influenced by several factors during SSF. The three major factors are biological factors, including microorganisms and substrates, physicochemical factors (moisture and water activity, temperature, pH, aeration and oxygen requirements, and particle size), and mechanical factors (agitation and mixing) (Cebrián & Ibarruri, 2022).

Physicochemical and biological factors

As SSF is defined as a process that occurs in the absence of free water, microorganisms able to grow at lower water activity (aw) values are the ones that best fit the process. In general, bacteria require higher values of aw for growth than fungi. Before fermentation, the substrate might need to be pre-treated such as cut, milled, or granulated to obtain the desired size, be moistened, or cooked to increase nutrient availability or be supplemented. Furthermore, the substrate may need to be sterilized or pasteurized outside or inside the bioreactor. Once the microorganism has been selected, the age and quantity of the inoculum are of vital importance, together with the cultivation medium and the physiological state. If the inoculum is not in the correct physiological state, a reduction of the final product will occur (López-Gómez *et al.*, 2020).

Temperature is one of the most critical factors to be controlled in SSF since changes during the process can cause from enzymatic inhibition to cell death, which can lead to a reduction in biomass and/or certain metabolites. During SSF processes, fungi metabolism can generate heat that must be removed from the system to avoid changes in substrate moisture content. Therefore, the main challenge is humidity control to prevent fungal growth reduction, while optimizing the mixing to combat fungal hyphae damage.

pH is another important factor during fermentation processes since it directly affects microbial growth. In general, filamentous fungi can grow in a wide pH range, making them suitable for growing in different agro-industrial substrates. Although pH variations can occur during the fermentation process due to metabolism, filamentous fungi can tolerate pH ranges from 2 to 9, with an optimal value of between 3.8 and 6.0. This ability can also be used to minimize bacterial contamination as they usually require a more restrictive pH control. On the other hand, the initial pH in the fermentation process is a critical factor since it influences the activities of enzymes involved in intracellular metabolism.

Particle size of the substrate affects respiration and nutrient availability in the process. A smaller particle size of the substrate increases the specific surface area for fungal growth, however, the interparticle space is reduced, decreasing aeration. Porosity is another important factor in SSF since it can affect gas diffusion.

Mechanical factors

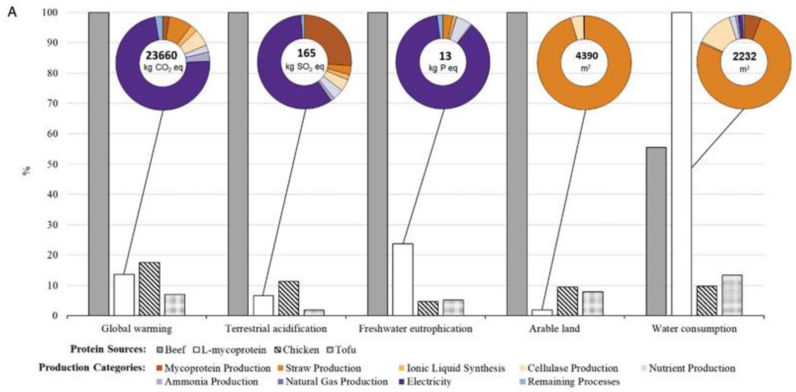
Oxygen requirement for SSF processes is a critical factor since filamentous fungi grow in aerobic conditions. Oxygen diffusion must be facilitated from the interparticle space to the biomass while carbon dioxide must be diffused from the biomass to the interparticle space. The main problem of the aeration rate is the depth of the substrate bed, which can limit oxygenation when it is not thin enough, with differences between reactor types. Mixing can add extra aeration; however, it can damage fungal hyphae. Forced aeration is another option supplying air that can be dry or humid. Dry air aeration provides better heat removal but reduces the moisture content. On the other hand, aeration with humid air does not reduce the moisture in the substrate bed. Mixing the substrate is important for heat removal, however, agitation of a bed that contains solid substrates with filamentous fungi growing on their surface could damage the fungus hyphae. Therefore, it is important to determine the frequency and intensity of the mixing, e.g. slower agitation speed and/or using humidified air(López-Gómez *et al.*, 2020).

Environmental impact of mycoprotein production based on LCA studies.

Some studies have evaluated the environmental impact of mycoprotein production based on life cycle assessment (LCA) methodology. Smetana et al. (2015) found that the production of 1kg of mycoprotein has a similar environmental impact than 1kg of chicken meat in endpoint damage assessment categories of human health, resources availability, and ecosystem quality. According to this study, when the comparison considers the calorific energy value or the content of digestible proteins, the results for mycoprotein are all worse than chicken and dairy-, insect-, gluten- and soy-based options. However, Mycoprotein can be considered an efficient meat alternative in the categories of land ($<2m^2$.year/kg) and water use (≈500L/kg) and performs similarly to the dairy product in energy consumption (15–20kWh/kg) (Smetana *et al.*, 2018). The use of agro-industrial waste can potentially reduce the environmental impact of mycoprotein production. For example, Upcraft *et al* (2021) investigated the environmental impact of mycoprotein production from Fusarium venenatum, a filamentous fungus, using rice straw as the substrate for mycoprotein production. Results indicated significant advantages in the impact categories of global warming, terrestrial acidification, freshwater eutrophication, and arable land use in comparison to beef production. However, the water consumption category for mycoprotein was higher because of the high-water demand of the rice culture. According to Jungbluth *et al.* (2016), the processing and distribution of mycoprotein has a high carbon footprint (4.99 kg CO_2eq. per portion). Similar or even higher rates of impact are found in Smetana *et al.* (2018), i.e., 5.55-6.15 kg CO_2eq. kg−1 and 60.07-76.8 MJ kg−1. Furthermore, recent studies defined the impact of 1 kg of protein (L-Mycoprotein) in the scope of 23.66 kg CO_2eq., 4.4 m^2 of arable land and 2.2 m^3 of water consumed (Upcraft *et al.*, 2021).

As shown in the study of Upcraft *et al*. (2021), lignocellulosic-mycoprotein delivers significant advantages over beef in all impact categories except water consumption and showed a similar environmental footprint to chicken on global warming and terrestrial acidification (Figure 10). However, in comparison to animal-sourced protein and plant-based protein (Tofu), lignocellulosic-mycoprotein shows significant environmental benefits in arable land-use. Thus, lignocellulosic mycoprotein offers a promising protein solution which is largely independent of arable land in comparison to land-demanding protein sourced from animal and arable crops (e.g. meat and tofu). The performance-limiting steps over the lignocellulosic mycoprotein life cycle vary between impact categories. Straw production dominates the impacts on water consumption (75.7%), due to rice irrigation. A less water-demanding feedstock would offer a mitigation solution to reduce water consumption. For global warming, total GHGs of 516 kg CO_2 eq. per tonne protein are attributed to process emissions. The main contributor is the emissions due to external electricity use and production representing 72.9% of emissions. Therefore, a potential decarbonisation solution would be to integrate renewable energy resources in lignocellulosic mycoprotein system to target over a third of energy-related emissions. For terrestrial acidification, the largest contributions to overall acidification are electricity and process emissions which contribute 58.4% and 26.3% respectively. In the case of marine eutrophication, 67.58% is due to straw production.

Although more expensive than meat-protein, Upcraft *et al*. (2021) demonstrated that lignocellulosic-mycoprotein offers significant potential for improving the sustainability of protein for human consumption. Lignocellulosic mycoprotein offers decarbonisation potential in comparison with protein derived from beef. However, the highlight of this technology is the minimum arable land usage required due to the main feedstock being agricultural residue. This research suggests that lignocellulosic mycoprotein sourced from food-safe lignocellulose represents a transformative solution to animal and plant-sourced proteins which are carbon-intensive and natural resource-demanding. Both animal-sourced and plant-sourced proteins not only cause significant environmental concerns on climate change and arable land use but also are constrained by long production cycles; thus, their supply chains are vulnerable to global arable land scarcity and extreme events e.g. public health emergency. In contrast, lignocellulosic mycoprotein not only enables decarbonised protein supply, which is largely independent of arable land but also offers future protein security due to its manufacturing in controlled environments and very short production cycles (Table 10).



Source: Upcraft *et al.*, 2021.

Figure 10. (A) LCIA comparison of lignocellulosic-mycoprotein (L-mycoprotein) vs. animal-sourced protein (beef and chicken) and plant-based protein (Tofu) on 5 impact categories; global warming, terrestrial acidification, freshwater eutrophication, arable land, and water consumption. LCIA results have been normalised to 100%. The breakdown of the process contribution to each impact category for lignocellulosic-mycoprotein is shown in the circular charts

Table 10. Overview of environmental requirements for cultivation of InnoProtein biomass for SCP production

Biomass	Water	Temperature	Energy	pH	Aeration	Growth rate	Substrate	Safety concerns	Waste products
Multicellular filamentous fungi	- Low compared to bacterial growth (SSF)	- Heat generated by fungi metabolism must be removed to avoid substrate moisture reduction and heterogenicity due to evaporation and condensation	- Low requirements for stirring and sterilization, no foam generation - Humidity control to prevent growth reduction -Optimize mixing to avoid hyphae damage	- Wide range for growth (from 2 to 9), optimal between 3.8 to 6.0 -Avoids bacterial contamination (restrictive pH) -Complex measurement (substrate particulate structure filled with a continuous gaseous phase)	- Growth in aerobic conditions -Particulate substrate facilitates O_2 and CO_2 transport -Depth of substrate bed (limiting factor) -By mixing or forced aeration	- Slow compared to bacteria (higher risk of contamination)	- Fungi can grow in simple, inexpensive substrates (e.g. Agricultural waste) - No need for solubilization of nutrients. - Need of substrate pre-treatment to obtained desired particle size/ moisture/increase nutrient availability/ sterilization -Particle size and porosity affect gas diffusion and nutrient availability	- Possible production of mycotoxins (process control and selection of strains)	- Low production of wastewater compared to submerged fermentation

Methylotrophic bacteria

Methylotrophic bacteria are a diverse group of microorganisms with many specialized enzymes that allow them to grow on low carbon substrates without carbon-carbon bonds and use them as energy sources. Methanol is a typical carbon substrate for many methylotrophic bacteria. Aerobic methylotrophic bacteria can use a CO_2-reduced carbon source. Methanol is one of the building blocks in the chemical industry and can be synthesized from petrochemical or renewable resources such as biogas. Considering that methanol can be produced by the conversion of methane from natural gas, the price of methanol has tended to decrease making it an attractive substrate in processes involving microorganisms to produce metabolites that add value to the bioprocess. Whitaker *et al*. (2017) suggested that the yield of a product obtained from methanol is like that obtained from glucose on a carbon-carbon basis. Methylotrophic bacterium bioprocessing technology has been studied in recent decades as it has been intended for the large-scale production of single-cell proteins. The InnoProtein project intends to optimize the growth of Methylomonas, Methylophillus and Methylobacterium as sources for proteins production.

Simões *et al*. (2022) studied different operational strategies for the growth of Methylobacterium on methanol as the sole carbon source and potassium nitrate as the only nitrogen source in a basic mineral medium. This study found that although *M. organophilum* was able to consume methanol at concentrations as high as 17 g/L, at methanol concentration up to 7 g/L all the substrate was consumed with the highest overall uptake rate of 0.25 g/(L·h). At this methanol concentration, the C:N ratio calculated was 19. The cell concentration corresponding to these growth conditions was 3.42g/L, 64% of the maximum cell concentration achieved in the study. A previous study performed by Jafari *et al.* (2008) found Methylobacterium that there is an optimum methanol concentration (18 g/L) for cell growth, and above this concentration, methanol inhibits cell growth. Methanol concentrations in the range of 10 to 12 g/L generated higher cell concentrations. Below this range, there was not enough carbon source to promote bacterial growth and above this range (>20 g/L). One important aspect to consider when cultivating these microorganisms is that methylotrophic bacteria growing aerobically on single-carbon (C1) substrates produce formaldehyde as a central intermediate, which is toxic to cells. Therefore, there should be a balance between the rate of methanol oxidation and the rate of formaldehyde assimilation to prevent cell toxicity.

In fed-batch cultivation, several aspects should be considered (Jafari *et al.*, 2008; Simões *et al*., 2022):

- Initial concentration of methanol
- Dissolved oxygen concentration in the reactor
- Aeration rate (L air min^{-1})
- Feeding time
- Cell yield
- Temperature (26-30 °C)
- Mixing (stirring)
- Retention time (60h)
- pH (7-8)

Previous research has shown that the use of this bacterium in pig feed (50% traditional meal and 50% SCP) and for aquaculture feed (replacing up to 52% of fishmeal in salmon farming) does not prevent growth or cause any damage to the nutritional profile of the product (Zhang *et al*., 2013). In addition, Tlusty *et al*. (2017) replaced up to 100% and 55% of the fishmeal in a compound feed for white shrimp and salmon, respectively, and tested their growth and consumer taste preference. The animals performed equivalently when they were fed with diets containing either the SCP from *Methylobacterium extorquens* or a standard aquaculture diet.

Methanol is a nonconventional substrate for microorganism growth; however, it has several advantages when used as a single-carbon substrate compared to methane, a gas which has a reduced solubility in a fermentation medium. A high percentage of crude protein can be found in *Methylobacterium organophilum* cells showed a high percentage of crude protein and the presence of almost all essential amino acids, corroborating the possible use of this microbial biomass as a single-cell protein. Furthermore, the production of carotenoids, exopolysaccharides and polyhydroxyalcanoates associated with *M. organophilum* growth makes this bioprocess commercially attractive (Simões *et al*., 2022).

Spiller *et al.* (2020) evaluated the environmental impact of microbial protein production on a food and beverage effluent as a substitute for soybean meal. Three different types of microbial protein (MP) production were included in the LCA, namely consortia containing Aerobic Heterotrophic Bacteria (AHB), Microalgae and AHB (MaB), and Purple Non-Sulphur Bacteria (PNSB). Results were compared against soybean meal production for the endpoint impact categories human health, ecosystems, and resources. Soybean meal showed up to 52% higher impact on human health and up to 87% higher impact on ecosystems than MP. However, energy-related aspects resulted in an 8-88% higher resource exploitation for MP. A comparison between the MP production systems showed that MaB performed best when considering ecosystems (between 13 and 14% better) and resource (between 71 and 80% better) impact categories, while AHB and PNSB had lower values for the impact category human health (8-12%).

Results shown in Figure 11 display that impacts on human health are mainly a function of climate change indicators except for MaB where most of the endpoint impact originates from particulate matter formation. For aerobic heterotrophic bacteria, the main contributors to ecosystem impacts are climate change and agricultural land occupation, whereas for soybean meal the agricultural land occupation, natural land transformation and climate change impacts have high contributions. Soybean meal shows a three times higher contribution in agricultural land occupation and nearly double the natural land transformation than microbial protein. Therefore, microbial protein production can be preferable to soybean production at large-scale (energy) optimized conditions. Results suggest that MP production generally has a high energy demand, reflected in all endpoint impact categories. being heat demand for drying and heating of reactors as well as electricity demand for harvesting the most energy-consuming processes (Smetana *et al*., 2017; Spiller *et al*., 2020).

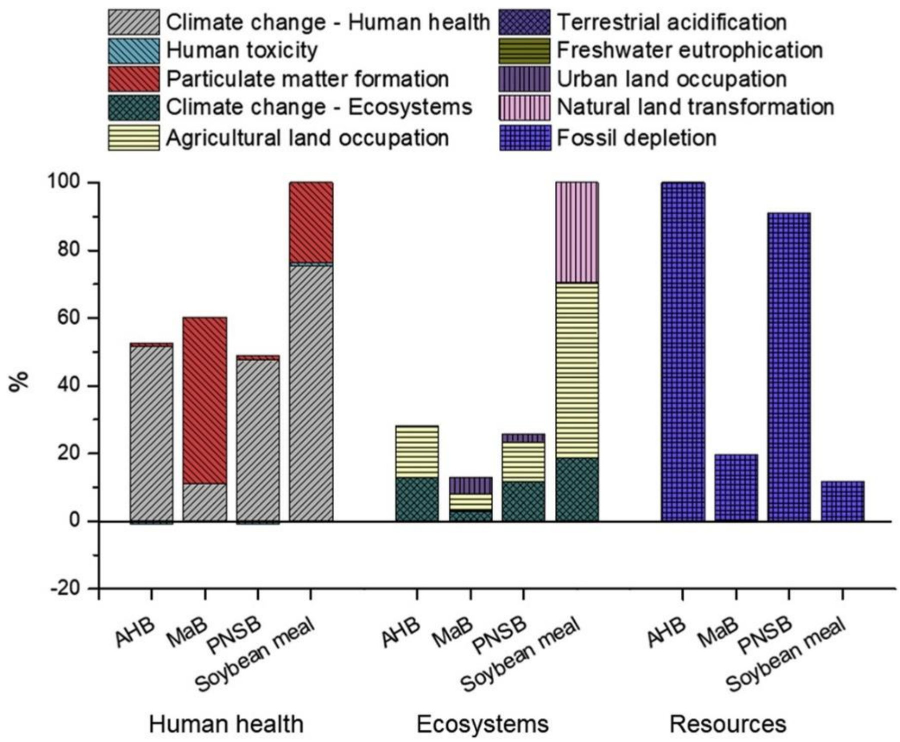


Figure 11. Contribution of individual midpoint categories to the endpoint impacts in terms of human health, ecosystems, and resource depletion. Source: (Spiller *et al.*, 2020)

3.3.1.3. Multi-insect system

Due to the increase in world population, the global food system is experiencing a crisis which is the forecast that the livestock sector is growing at a rate deemed unsustainable (Pelletier, 2010). Based on the above, it is necessary to find alternative sources of protein that can be produced sustainably, and in recent years edible insects have been proposed as one potential 'new' protein source. Insects have been the subject of recent studies as a potentially environmentally sustainable and nutritious alternative to traditional protein sources because many of them can be farmed at relatively low economic and environmental costs (Pelletier, 2010).

Based on the literature, it is found that farming insects use up to 50–90% less land per kg protein, 40–80% less feed per kg edible weight and produce 1,000–2,700g fewer GHGEs (Greenhouse gas emissions) per kg mass gain than conventional livestock (Vantomme, 2014). However, particularly in Europe, insects are a new food and information about the safety and nutritional value of edible insects is scarce, particularly since they are such a diverse category (Rumpold, 2013).

A study of comparison using edible insects and commonly consumed meats using two nutrient profiling models developed to combat over and undernutrition, concluded that insect nutritional composition is highly diverse in comparison with consumed meats. The food category 'insects' contains some foods that could potentially exacerbate diet-related public health problems related to over-nutrition but may be effective in combating under-nutrition (Payne, 2016).

The current food systems are facing several sustainability problems. One major issue is the environmental impact of food production, which contributes to climate change, deforestation, and biodiversity loss (Poore, 2018). Additionally, current food systems depend on finite resources, such as water and fossil fuels, which are becoming increasingly scarce. Moreover, the current food systems have a significantly negative impact on human health, particularly the increasing incidence of chronic diseases linked to the overconsumption of animal products (Willett, 2013). Furthermore, the current food systems are inequitable, as they often fail to provide adequate access to food for marginalized communities and contribute to social and economic inequality (United Nations, 2019). As a result, it is crucial to develop sustainable food systems that can provide nutritious food for a growing population while minimizing negative environmental and social impacts (Smetana *et al*., 2023).

Alternative proteins, such as plant-based, lab-grown meat and insects, can potentially address many of the sustainability issues associated with current food systems. For example, replacing animal-based products with plant-based alternatives has significantly reduced greenhouse gas emissions, land use, and water use associated with food production (Poore, 2018). Insects are also seen as a promising alternative protein source, they have a high nutritional value, are rich in protein, vitamins, and minerals, and have a lower environmental impact than traditional livestock (Van Huis, 2013).

Furthermore, alternative proteins can contribute to food security by reducing dependency on finite resources and increasing the resilience of food Systems (Sexton *et al*., 2019). Alternative proteins can also improve human health by providing nutritious food options and reducing the incidence of chronic diseases linked to the overconsumption of animal products (Willett, 2013).

Environmental pollution, population increase, water availability and misuse of land are inexorably driving humans to take on important challenges related to sustainability. The future is expected to see a significant increase in food and feed demands, which poses a serious threat to the well-being levels and even the survival of modern societies. Within this scenario, the efficient and sustainable use of insects as protein sources has been invoked as a possible strategic solution. As a candidate for remarkable growth, insect farming promises significant benefits to the agri-food industry, offering interesting opportunities for implementing a circular economy (Cadinu *et al*., 2020).

It is well-known in the scientific community regarding the benefits of the use of insects for nutrition purposes. On the one hand, insects are everywhere. They can be found in abundance and can be grown across the world. On the other hand, they are quite robust, overall, and, at least within a wide range of conditions, adapt easily to different environmental conditions. Furthermore, insects have short reproduction cycles, which translates into a quick biomass build-up. They also exhibit a remarkably high growth rate. This mirrors a very high efficiency in converting feed mass into body mass (Cadinu *et al*., 2020). It is how the feed conversion efficiency of insects is much higher than that of mammalian livestock. Despite their intrinsic variability related to specific case studies, quantitative investigations indicate that, on average, livestock must be fed about 6 kg of plant proteins to produce 1 kg of high-quality animal proteins (Van Huis, 2013).

Another aspect to consider is that the entire life cycle of insects is characterized by an extremely reduced environmental footprint compared with that of common livestock. Greenhouse gas emissions are significantly lower. While 18% of total greenhouse gas emissions can be ascribed to the livestock sector, including direct and indirect emissions for livestock rearing, emissions per year related to insects are more than 100 times Lower. The comparison of five insect species, pigs, and beef cattle, in terms of CO_2 production per kilogram of mass gain, clearly shows that insect rearing can be extremely advantageous. While beef cattle produce, on average, 2,850 kg of CO_2 per kilogram of mass gain and pigs up to 1130 CO_2 per kilogram of mass gain, Pachnoda marginata, Blaptica dubia, Locusta migratoria, Tenebrio molitor and Acheta domesticus produce, respectively, 122, 38, 18, 6 and 2 CO_2 per kilogram of mass gain. Concerning organic wastes that can contribute to environmental pollution, at least a tenfold difference between livestock and insects has been estimated (Oonincx *et al*., 2010).

Water consumption is another advantage to be considered with the use of insects. Simple calculations suggest that the production of 1 kg of animal proteins requires up to 20 times more water than the production of 1 kg of grain protein (Chapagain; Hoekstra, 2003). However, the amount of water to be used to obtain 1 kg of meat could become as high as 100 times larger, if the water needed to grow forage is considered. Overall, it appears that about 2,300 L of water are needed to produce 1 kg of proteins from chickens, but the figure increases up to 3,500 L for 1 kg of protein from pigs and to 22,000 L to obtain 1 kg of proteins from cows. For the latter case, different estimates have been proposed that make the amount of water required equal to no less than 43,000 L (Pimentel *et al*., 2004).

Regarding land use, studies also suggest that insects, and mealworms in particular, require much less land than other sources of animal proteins. It has been estimated that the amount of animal proteins produced from mealworms grown in 1 ha of land requires 2.5 ha of land if animal proteins are obtained from milk, up to 3.5 ha in the case of poultry and pigs, and no less than 10 ha in the case of cattle (Oonincx; de Boer, 2012).

Finally, it is worth mentioning that several insect species exhibit the capability of converting efficiently organic wastes, side-streams, and by-products of agricultural processes to animal proteins. The process is known as the bio-conversion process, and it is attracting increasing interest as a valid alternative to more traditional methods of treating organic wastes. It is precisely intensive insect rearing that offers the unique opportunity to break down significant amounts of organic wastes, side streams and by-products while increasing the total insect biomass and producing a range of products from biofuels and fertilizers to fine chemicals for the pharmaceutical industry. The method is particularly advantageous in terms of sustainability because it relies upon the use of organic wastes as the substrate for the intensive production of insect biomass. In this way, insects become active agents in the waste management process, turning large amounts of organic waste destined for disposal into valuable products. For this reason, bioconversion is receiving increasing attention from both researchers and entrepreneurs. At present, however, only a few studies are available, mostly focusing on a handful of species belonging to the Diptera and Coleoptera orders. It follows that great opportunities exist to investigate the subject and identify the best combinations of insects and organic wastes that allow for maximizing both bioconversion and insect farming processes (Fowley; Nansen, 2020).

Insect production methods

Although insect rearing looks set for a brilliant future, little information is available concerning insect production methods and technologies, especially on a large scale. One of the reasons lies in the understandable reluctance of private companies to share details involving the heart of their business. A few patents are available, but they do not disclose sensitive data and performances. In contrast, much more is known about traditional rearing methods, which represent the background for any technological improvement (Cadinu *et al*., 2020).

In general, insect farming involves at least two main separate units, namely, one for the maintenance of the breeding colonies in captivity and the other for growing larvae from eggs. If the business focuses on mature insects, an additional rearing area is needed. Advanced systems often include an area to process insects and refine derived products. Production wastes, such as substrate leftovers and frass, can be used to produce fertilizers in a dedicated facility, thus contributing to circularity and sustainability (Cadinu *et al*., 2020).

Insects show the considerable advantage of thriving in densely populated areas, which allows intensive production even in confined spaces. Usually, larvae and pupae are placed together with a feeding substrate in small trays that can be made of different materials such as wood, high-density polyethylene, or fibreglass. Trays used for fattening Tenebrio Molitor (TM) larvae a standard 65×50×15 cm^3 boxes, easy to handle and deep enough to prevent larvae or adults from escaping (Dossey et al., 2016). A recent study based on an EU pilot mill designed to produce 17 tons of fresh TM larvae per year reports that mealworms can be reared with a density of 5 larvae cm-2 (Thévenot *et al*., 2018).

As an example, the DESIRABLE project (DESIgning the Insect bioRefinery to contribute to a more sustainABLE agro-food industry), funded by the French National Research Agency (ANR), has provided a few interesting case studies on the practical organization of a medium-scale (400 tons per year), large-scale (2,000 tons per year), and very large-scale (10,000 tons per year) biorefinery of TM larvae. About 13,000 boxes can be placed in a 400 m^2 building, leading to a production of about 250 kg of larvae m-2 per year. At the end of the fattening stage, larvae are harvested and processed to obtain the final insect meal (Halloran *et al*., 2018).

Rearing conditions

The efficiency of insect farms in terms of feed conversion strictly depends on rearing conditions. Insects do not have metabolic regulation of their body temperature. Therefore, suitable temperature and humidity must be ensured to optimize insect growth depending on the species and growth stage. Typically, temperatures range between 20 and 35°C and humidity levels range from 55% to 75%. Air must be circulated to avoid undesired air stratification, the proliferation of fungi, bacteria or viruses, and the accumulation of CO_2 and other dangerous gases that can have detrimental effects on the health of the colony and workers (Dossey *et al*., 2016).

Climate control is particularly important for at least two reasons. First, even temperature differences of 2 or 3°C can lead to significant time shifts in insect development, thus resulting in uncontrolled farming outcomes. Second, the climate control system contributes to the energy demand, especially for insect farms located in temperate countries with cold winters and warm summers, where heating during winter and cooling during summer are required. In this regard, an innovative system was recently developed to separately monitor a small cluster of cages, thus enabling more accurate and efficient climate control (Climate Regulation System for Insect Breeding, 2020).

Insect processing

The full production from adults to insect meal involves several steps that, in turn, require employees. Concerning the farrow-to-wean area, efforts are mainly related to feeding adults, removing the dead and frass, monitoring oviposition, and releasing new adults into the colony. Eggs must be periodically collected and moved to the rearing area after they hatch. Here, the new larvae are in trays with the proper amount of substrate and water. Concerning the fattening area, larvae must be periodically moved to clean trays with new substrate and water. Finally, the larvae that have reached the desired size must be removed and processed. During this stage, larvae that have undergone slower growth can be separated and moved to the subsequent batch (Dossey *et al*., 2016).

A common pretreatment used in the commercialization of insects and related products is blanching. It consists of a short boiling step followed by rapid cooling in flowing cold water to reduce microbial counts and to inactivate the enzymes responsible for spoilage and food poisoning. However, blanching is ineffective on mesophilic bacterial spores and a further drying process to reduce the water content is needed (Cadinu *et al*., 2020).

Several technologies such as sun drying, freeze drying, microwave drying, smoke drying and oven drying can be applied to insect manufacturing. Other methods are often used to ensure a better ratio of macronutrients in the final insect feed, e.g., to reduce lipid or chitin contents, or to obtain derived products such as oil and protein powders and pellets. This allows for more effective risk management (Cadinu *et al*., 2020).

Challenges

Despite the remarkable interest attracted in recent years and the impressive work of research and analysis carried out by a few scientists, insect rearing for food and feed production is still in its infancy. It represents one of those rare fields of study in which origins and evolution make fundamental and applied research extremely close to engineering and industrial application (Cadinu *et al*., 2020).

Insects can be part of a rational and profitable solution to meet the food and feed demands in the future. However, the use of insects as food and feed sources brings with it several problems on different levels. Some of them are discernible today, but it is likely that other issues will emerge as the field of study evolves further and insect farming becomes a more common practice (Cadinu *et al*., 2020).

The first main challenge that deserves mention is related to the acceptance of insects and insect-based products in modern societies. Although edible insects have been part of the human diet in various regions around the globe since ancient times, people living in modern societies, particularly in Western countries, feel uncomfortable with the idea of consuming food with ingredients deriving from insects (Cadinu *et al*., 2020).

The second main challenge concerns the accurate evaluation of the factors involved in insect rearing. The transition from the traditional wild catch of edible insects from their habitats to massive farming requires careful study. The subject is extremely broad and only a truly multidisciplinary approach can assure significant progress in a relatively short time. Specific expertise is needed to advance knowledge regarding the effects of rearing conditions and insect diet on the insect's health, its growth rate in the different growth stages, its nutritional profile, and its capability of converting agricultural wastes and by-products into useful biomass (Cadinu *et al*., 2020).

The third main challenge involves the intensive insect rearing itself. A fair balance between mechanization, automation, and manual labour must be found. Most of the insect farming methods utilized are labour-intensive. Only a few stages are automated, which makes a huge staff necessary when rearing scales up. Therefore, products available on the market are still overpriced compared with other feed sources such as soy meal (Cadinu *et al*., 2020).

Life cycle sustainability assessment

The underlying concept of using insects as a novel protein source in feedstuffs has great utility potential. However, the environmental impact and socio-economic performance of production processes and products of that origin remain widely unexplored. In aiming for sustainable production and consumption patterns, consideration must be given to the environmental, economic, and social implications (PROteINSECT, 2016).

To design sustainable insect production systems that are suitable for adoption by small and large-scale operations in different regions of the world, WP4 will perform comparative Life Cycle Assessments on a wide range of production systems in different biophysical and socio-economic environments. The concept of Life Cycle Assessment is based on an evaluation of the impacts of products and services over their complete life cycle, i.e. from extraction of raw materials, transport, processing and assembly to distribution, end use, and waste disposal. To address all sustainability dimensions, we will employ different life cycle methods including environmental Life Cycle Assessment (env. LCA), Life Cycle Costing (LCC) and Social Life Cycle Assessment (S-LCA). Apart from making informed choices on products, Life Cycle Thinking methodologies are particularly suitable to detect efficiency deficits and yet untapped improvement potentials and thus are highly relevant for product development and consultations towards policy change (PROteINSECT, 2016).

To guarantee comparability with other protein-supplying feedstuffs, the evaluation will be made on the functional utility of 1 kg of crude protein for relevant livestock. The nutritional performance and ileal digestibility of 1 kg of insect-borne crude protein will be evaluated in respective feeding trials.

These comprehensive and informed LCA analyses will yield information that supports decision-makers on customized measures that ease the implementation of insect proteins for feed while countering yet unforeseen negative externalities (PROteINSECT, 2016).

While life cycle assessment (LCA) studies and other specific information about insect production can provide valuable insights into the environmental and nutritional aspects of insect-based food systems, it is important to note that these studies do not provide a comprehensive understanding of the sustainability potential of insect production chains on a European level. Factors such as social acceptability, economic feasibility, and production scalability are also crucial to consider when evaluating the sustainability of insect production chains. Additionally, the results of LCA studies on insect production can be affected by several variables, such as insect species, the type of feed used, and the production method, which can lead to varying results. Therefore, it is important to consider a wide range of sustainability indicators, including social, economic, and environmental aspects, to fully evaluate the potential of insect production as a sustainable food system on the European level (Smetana *et al*., 2023).

This study relied on the FAO Sustainability Assessment of Food and Agriculture Systems (SAFA) guidelines to analyse published data on environmental indicators such as greenhouse gas emissions, land use, water use, biodiversity, energy, and animal welfare (FAO, 2014).

SAFA is a comprehensive worldwide framework that evaluates sustainability across food and agriculture value chains. It serves as a universal benchmark for analysing the interplay between various sustainability dimensions and identifying conflicts and opportunities for mutual benefits. By assessing these indicators, this study provides a holistic basis for the identification of the potential of insect production to tackle environmental hotspots of sustainable food systems on the European level (Smetana *et al*., 2023).

SAFA concentrates on supply chains and enterprise(s) as elements of those chains. The LCA approach focuses on the evaluation of the environmental impacts of a product through its lifecycle, and, therefore, is not always suitable for the sustainability analysis of regions and countries. Similarly, to LCA, SAFA covers multiple components of inputs, outputs, and environmental impacts; however, its focus on a larger system scale (value chains) enables a more comprehensive consideration of the scope of good governance and social well-being of sustainability (SAFA) (Smetana *et al*., 2023).

Atmosphere

Impacts associated with GHG emissions in insect production systems depend heavily on the use of diet. Thus, using a standard diet based on commercial or proprietary feed is associated with 2.3–3.1 kg CO_2eq per kg of fresh insects produced (Oonincx and de Boer, 2012; Halloran *et al*., 2017). This aligns with the results found for 1 kg of dried larvae, which is 5.76 kg CO_2eq (Bava *et al*., 2019), and for 1 kg of protein, which is 3.9–7 kg CO_2eq (Halloran *et al*., 2017; Bosch *et al*., 2019). However, some studies have reported a higher carbon footprint of up to 21.1 kg CO_2eq per kg of fresh larvae (Suckling *et al*., 2020) or 15–29 kg CO_2eq per kg of protein (Ulmer *et al*., 2020) when the production systems are specific and so on not optimized for the production. These higher impacts can be attributed to the inclusion of frass application to the field as an emission factor (Suckling *et al*., 2020) or the analysis of a different production system with a low technology readiness level (Ulmer *et al*., 2020).

Water footprint

It should be noted that water footprint is only indicated in a limited number of studies, with insects grown on a control diet resulting in 0.42–0.82 m^3 of water depleted per 1 kg of fresh insects (Halloran *et al*., 2017; Suckling *et al*., 2020). The same impact is found for the protein-based unit at 0.71 m^3 (Halloran *et al*., 2017). When calculated based on dry matter content, the impact increases to 1.26 m^3 (Bava *et al*., 2019). The production of insects on by-products (food waste) results in varying levels of water depletion, from a low of 0.8–1.1 m^3 per kg of dry matter content (Bava *et al*., 2019) to a high of 8.5–11 m^3 per kg of fresh insects produced (Roffeis *et al., 2017*). The water footprint of insects produced on manure is also inconsistent, with ranges from a low of 8.5–11 m^3 per 1 kg of insect on a dry matter basis (Roffeis *et al., 2017*) to a very high of 113.9–187.6 m^3 (Roffeis *et al., 2015*). There is a lack of studies evaluating the water footprint of insects grown on food waste and manure.

Land use and biodiversity

Despite the development of novel biodiversity assessment approaches, most LCA studies still rely on the land use category as a representation of the mentioned aspects. Land use of insect production indicated in studies in a wide range of impacts from 3.6 m^2 per kg of fresh insects (Oonincx and de Boer, 2012) to as high as 94.7 m^2 per 1 kg of insects on a dry matter basis (Bava et al., 2019) and 1.1–93 m^2 per 1 kg of proteins (Bosch *et al*., 2019; Ulmer *et al*., 2020). Using by-products and wastes in the feed of the insects should lower the impacts to 1.6 m^2 per 1 kg of fresh insects produced (Thévenot et al., 2018); -16.8 to 7.7 m^2 per 1 kg of insect on a dry matter basis (Roffeis et al., 2015; Bava *et al*., 2019; Smetana *et al*., 2019; Ites *et al*., 2020) and 0–1 m^2 per 1 kg proteins (Bosch *et al*., 2019).

Material and energy use

Material and energy use is an important factor influencing the sustainability of food systems. As previously mentioned, factors in the energy use of insect production chains depend on the types of diets used (Smetana *et al*., 2021). Insect production on conventional diets results in energy use of 33.7 MJ per 1 kg of fresh insects (Oonincx and de Boer, 2012) and 159–425 MJ for 1 kg of proteins (Bosch *et al*., 2019 and Ulmer *et al*., 2020). Energy use for insect production, when grown on by-products and waste, is highly varied and ranges in the scope of -108 to 62.8 MJ per 1 kg of insect biomass on a dry matter basis (Roffeis *et al*., 2017; Thévenot *et al*., 2018; Ites *et al*., 2020) or 18–77 MJ per 1 kg protein (Bosch *et al*., 2019).

Animal welfare

The animal welfare implications of insect production for food and feed in the European Union (EU) have not been extensively studied. However, a study suggests that compared to traditional livestock production, insect farming can provide a more humane environment for the insects, as they can be reared in smaller spaces, with less stress and better access to food. The humane environment is not the only ethical factor, and among others indicated for insects are environmental impact, human and animal health, human preferences and social acceptability, animal welfare, and broader animal ethics issues. The ethical issues related to animal integrity, death, and naturalness have been extensively studied in livestock farming and aquaculture for many years. Established considerations and rules should be reevaluated in the context of insects and insect rearing, even though the concept of death and integrity, and even the phenomenological analysis of the possibility of experiencing empathy towards insects as a basis for including insects in the moral community, may seem unusual at first glance. Nevertheless, some authors point out that causing death is worthy of ethical consideration in and of itself, regardless of whether the animal suffered now of killing. Taking the death considerations in the life cycle assessment perspective, some proposed approaches count for the number of animals needed to deliver the same function (e.g., the amount of food).

3.3.2. Protein recovery

3.3.2.1. Three-phase partitioning technology (TPP)

The traditional methods for protein purification are intricate, and ongoing process development aims to enhance purity, yields, and cost-effectiveness while minimising processing time. The analysis of enzyme behaviour in anhydrous media holds significant implications for biotechnology and bioorganic chemistry, yet current understanding of the interactions between proteins and organic solvents is limited (Singh *et al.*,2001). A comprehensive understanding of these interactions could open avenues for various applications, including organic synthesis, biosensors, bioseparation, and potentially enhancing enzyme activity and stability.

A novel bioseparation strategy, known as Three Phase Partitioning (TPP), proves valuable both upstream and downstream in enzyme protein production. Physico-chemical factors such as kosmotropy, electrostatic forces, conformation tightening, and shifts in protein hydration have been proposed as the basis for proteins emerging as insoluble phases in TPP (Dennison & Lovrien, 1997).

TPP stands out as a cost-effective and efficient single-step process for separations, showcasing remarkable potential as an alternative to conventional methods in protein recovery. However, challenges arise during the scaling up and engineering phases of TPP extraction concerning factors such as the solvent type, degree of mixing, and protein characteristics in large-scale processing. While this method can be adapted for use in commercially available equipment, some modifications are necessary to achieve improved flow uniformity and prevent issues like supersaturation or inadequate distribution (Gagaoua & Hafid, 2016).

Resource consumption

The industrial application of TPP will involve substantial usage of raw materials like partitioning agents, solvents, and other extraction materials, potentially leading to significant expenses. Additionally, energy is utilized at various stages of TPP, including mixing, extraction, and separation. Water is frequently incorporated into TPP, and its consumption varies depending on the application and process conditions. To optimize resource usage in TPP, it is common to implement sustainable practices. These practices encompass selecting environmentally friendly solvents and chemicals, employing energy-efficient equipment and technologies, adopting recycling and reuse strategies for raw materials, and enhancing process efficiency to minimize overall resource demands (Chew *et al*., 2018).

Waste generation

Nevertheless, extensive processing on a large scale will result in a substantial volume of waste chemicals that must be efficiently managed, particularly through recycling. Further investigation is warranted to explore the recycling possibilities of polymer-bound ligands, allowing for their reuse in subsequent affinity extraction processes. The industrial potential of TPP can be increased by reducing the waste volume generated through recycling of the phase components (Chew *et al*., 2018):

- The use of solvents and chemicals in the partitioning process may result in chemical waste. Disposal of these substances requires careful consideration to avoid environmental contamination.
- The process can produce by-products or residues from the extraction and partitioning steps. These may include substances that need proper management to prevent adverse environmental impacts.
- Unused partitioning agents or reagents might be present in the waste stream, contributing to the overall waste generated during TPP.
- Residues from the extraction process, including remnants of the polymer-bound ligands or other extraction materials, may contribute to the waste generated.

- Depending on the application, biological waste may be generated if microorganisms or cells are used in the TPP process.
- Water consumption and potential contamination during the process can result in water waste that requires proper handling.

Chemical use

T-butanol has been a predominant choice in nearly all previous applications of TPP. However, a notable concern associated with the extensive implementation of TPP on a large scale is the utilization of significant quantities of t-butanol. T-butanol shares similarities with ethanol in terms of its flash point and volatility (Przybycien *et al*., 2004). Moreover, t-butanol poses certain risks, including acute toxicity, irritation to the eyes and skin, as well as symptoms such as nausea and dizziness (Chew *et al*., 2018).

3.3.2.2. UAE: Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) is an innovative technology for the recovery of bioactive compounds. It is based on the application of ultrasonic waves to a matrix immersed on a liquid medium, producing the rupture of cell walls and releasing the compounds of interest (Chemat and Vorobiev, 2019). High extraction yields can be achieved with this technology, without interfering with the integrity of bioactive compounds since temperature is controlled through all the extraction process. Furthermore, it is considered a green technology since and it requires low solvent volumes, short extraction time and is energetically efficient. It can also be combined with other extraction technologies such as microwave assisted extraction (MAE), maceration, among others (Carreira-Casais *et al*., 2021) .

UAE for protein extraction

Many studies utilising UAE for obtaining proteins from algae have employed eco-friendly solvents, predominantly water. Extraction times typically ranged from 1 to 2 hours, with lower temperatures commonly utilized, reaching high protein recovery from algal sources compared to other techniques such as MAE. Consequently, these procedures align with the principles of green chemistry. It is crucial to highlight the significance of pH and the other parameters shown in Table 11, in the extraction process, as they significantly influence protein extraction and isolation. In general, a neutral pH and temperatures around 45 °C favours protein extraction using water as solvent, whereas, low pH ranges are conducive to protein isolation (Carreira-Casais *et al*., 2021).

Table 11. Main principles and operational parameters affecting UAE

Main principle	Physical parameters	Reactor parameters
Acoustic cavitation Disruption of cellular matrix by shock waves and microjets Increased mass transfer by turbulence and acoustic streaming Reduction in particle size to increase surface area	Frequency: Intensity/power/amplitude	Selection (bath or probe) Size and shape Pressure control Temperature control Irradiation mode

Source: Wen *et al.*, 2020

UAE combined with other techniques

Conventional extraction techniques can be combined with UAE to mainly reduce extraction time (ET) by pursuing a mechanical effect, which allow greater penetration of solvent into the sample matrix and increase the contact surface area between the solid and solvent. The decrease of the ET without modifying the molecular structure and molar mass distribution is one of the main advantages that favours protein recovery (Table 12).

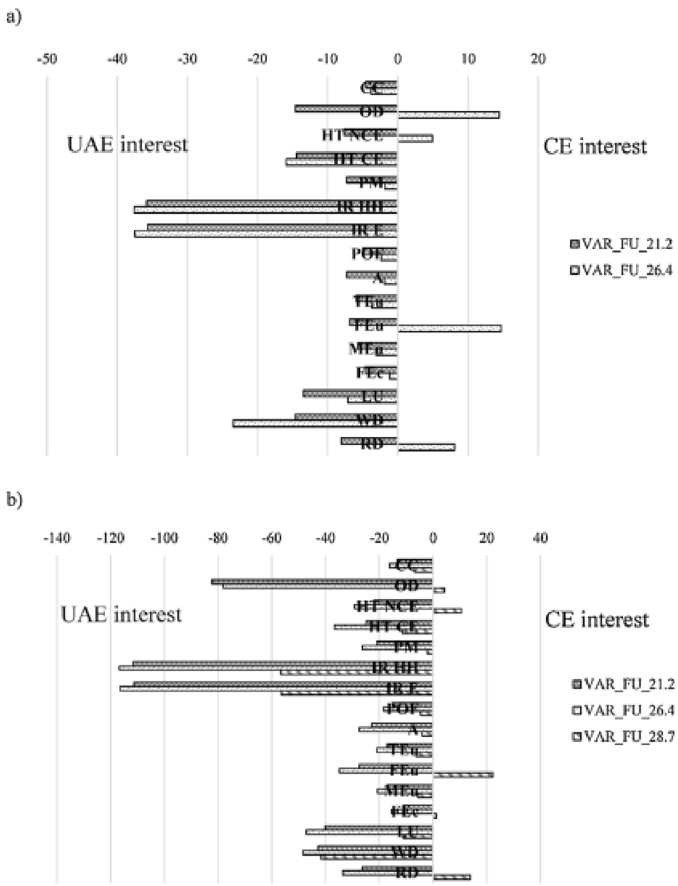
Table 12. Comparison of the efficiency of UAE and other techniques in the recovery of algae protein

Extraction techniques	Conditions	Recovery (%)
UAE	Water, room temperature, 60 min	84
Alkali extraction	1 M NaOH, room temperature, 15 min	75
EAE	NaH_2PO_3 buffer, 1% cellulase, 50˚C, 180 min	81
Thermal extraction	120 ˚C, 5 min	64
MAE	Water, 1000 W, 3 min	79

Source: Mahali and Sibi (2015).

Environmental impact of UAE

The use of UAE for the recovery of proteins is an accessible and easy to work technique that, provides high-quality performance of the extraction process, without the use of large amounts of chemical reagents and energy. UAE involves energy, solvent, and time savings, which have positive implications not only on process productivity and cost reduction, but also on environmental impacts. An LCA study performed by Carciochi *et al.* (2021) reported that UAE has lower environmental impacts than conventional extraction (CE) techniques. This Study showed that UAE provided significant enhancement of extraction yields and/or kinetics of the process compared to CE, depending on extraction temperature. Figure 12 compares CE and UAE at 70◦C showing the variations for the 16 impact categories. The results confirmed the clear advantage of UAE at 70◦C, due to faster extraction and much lower energy consumption during UAE. As a whole, the use of UAE significantly reduced environmental impacts of extraction processes.



Source: Carciochi *et al*. 2021.

Figure 12. Variation of impact categories between UAE and CE for the processes developed at a)50˚C and b)70˚C. Source: (Carciochi *et al.*, 2021)

3.3.2.3. ASE and NADES extraction technologies

Proteins are one of the three essential macronutrients in the diet and the most important to prevent malnutrition and hunger (Chou *et al*., 2012). According to the United Nations, the global population will increase to 9.5 billion people by 2050 (UN, 2022); as a result, the demand for food, and therefore also for protein, will rise (Shahid *et al*., 2020). There are two primary protein sources of similar nutritional quality: animal (which includes meat, fish, eggs, and dairy products) and plant (legumes, and to a lesser extent, seeds, and nuts). However, animal protein is known for its high environmental and climate impact, not only for the emissions related to their production but also due to the arable land used for the intensive crop production for feed and a large amount of water required (for both feed and animals) (Röös; Bajželj; Smith *et al*., 2017). Thus, it is necessary to find sustainable plant-based sources and production processes to meet future protein requirements without damaging the planet and human health (Naseri *et al*., 2020). One of the most promising alternative feedstocks is microalgae, which are photosynthetic organisms mainly composed of proteins, carbohydrates, and lipids.

This type of biomass has several advantages over others, including superior photosynthetic efficiencies and biomass production potentials than terrestrial crops, growth over a wide range of pH and temperature, capacity to uptake nutrients from different types of sources, such as wastewater, and most of them have a short generation cycle (Karemore & Sen., 2016).

The characteristics and composition of microalgae depend on the species and the environmental and operational conditions during cultivation (Shahid *et al*., 2020). However, in most cases, the main constituents of microalgae biomass are proteins, ranging from 30 to 70% mass percentage in dry weight, achieving higher protein concentrations in microalgae grown in stressful environments such as wastewater. Due to the proteins' high added value and liability, it is reasonable to first recover the protein fraction in the process of fractional valorisation of this biomass (Moldes *et al*., 2022). The major issue with protein recovery is related to where the proteins are found in the cell. Typically, these biomolecules are inside a very recalcitrant cell wall that is difficult to break and whose thickness and chemical composition depend on various factors such as growth stage, cellular form, and environmental conditions (Córdova & Chamy., 2020).

The use of organic solvents is a well-known technique for the extraction and separation of microalgae biomolecules, but some of them are toxic and not aligned with environmental and human health concerns (Corrêa *et al.,* 2021). Alternative solvents recently developed can be used to optimise the protein extraction from microalgae processing with lower environmental impact than conventional solvents, those are Ionic Liquids (ILs) and Deep Eutectic Solvents (DESs).

Protein recovery

One of the bottlenecks of the protein extraction process from microalgae is the recovery, as the purity levels of the proteins obtained will determine their subsequent applications. Generally, using a single recovery step is not enough to isolate proteins of enough purity. Thus, the current trend is to follow a multiple-step approach (Grossmann *et al*., 2020). The process becomes complicated because of the hydrophilic nature of proteins, which makes them prone to denaturation, precipitation, and degradation. Proteins are fragile molecules, so mild operation conditions are required to preserve their native structure and biological activity.

There are conventional separation techniques such as precipitation methods, membrane separations, electrophoresis and chromatographic techniques, and new emerging alternatives such as biodegradable solvents, which are more efficient due to protein purification. Other techniques such as molecular imprinting, magnetic separation, or electrostatic separation have been attracting scientific interest, but they are still in an incipient state.

Solvent extraction

Solvents can be used to release the proteins from inside the biomass cell, but they can also be employed to selectively obtain proteins from the released compounds through conventional liquid–liquid extraction (LLE). This separation technique consists of the analyte's migration to the extracting phase, relying on the different affinity for the medium (measured by the distribution constant, KD) and the concentration gradient (Moldes *et al*., 2022).

Conventional solvents for protein recovery are hydrocarbons, alkalis, acetone, alcohols, and chloroalkanes. Despite these solvents being low-cost and having good solubility capacities, they also present important drawbacks such as long operational times, high solvents and energy consumption, and the high odds of separated protein damage. Furthermore, most of them are volatile organic compounds (VOCs) derived from non-renewable sources such as petroleum, being both detrimental to human health and the earth's well-being (Chemat *et al*., 2019).

Due to the drawbacks of classical solvents, alternative strategies are needed to achieve more sustainable processes, with reduced energy consumption and lower presence of hazardous substances. One approach to accomplish these goals consists of replacing classical with green solvents, which must be cheap, non-toxic, safe, stable, biodegradable, renewable, and atom-economic in their synthesis; however, telling which solvents are green is still a controversial issue (Moldes *et al*., 2022). The biodegradable solvents are one of the greener solvent options for LLE, which are not necessarily fully green (although every green solvent must be biodegradable). These solvents can be combined with innovative assisted technologies such as microwave, or ultrasound, to achieve an optimum purification Process and this is the case for ILs and DESs (Moldes *et al.,* 2022).

Biodegradable solvents for protein extraction

The two types of biodegradable solvents more studied for biocompounds recovery, and in particular for proteins, are ILs and DESs, which are also known as "designer solvents" because their physicochemical properties can be tuned if needed for further improvements on the separation performance, achieving high protein recovery yields (Chen & Wang., 2010). Most authors do not grant them the category of "green" because, despite their good biodegradability and environmental advantages, other requisites needed are not fulfilled, such as high stability under high temperature and low pressure, flammability, and potential toxicity (Chen & Mu., 2021). In addition, other aspects related to solvent preparation must be examined, for example, the synthesis process of ILs employs organic solvents and demands high temperatures and reaction times, not to mention the final purification step always required, so overall, the process could not be considered green (Benvenutti *et al*., 2019).

Deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs)

To avoid the problems associated with ILs, scientists turned their interest to a newly emerging type of solvent, DESs, which was first described in 2002 (Abbott *et al*., 2002). DESs are liquid mixtures at room temperature, formed by the combination of two solids (or a solid and a liquid) in a specific molar ratio, for which the eutectic point temperature is below that of an ideal mixture, much lower than the compounds separately (Martins, *et al*., 2019). DESs have two components: a hydrogen bond donor (HBD) or a metal halide and a hydrogen bond acceptor (HBA). The hydrogen bond interaction between those components forms the eutectic mixture (Moldes *et al*., 2022). The variety of those two groups is enormous, so it is estimated there could be around 10^6–10^8 possible combinations to form DESs (Beyersdorff *et al*., 2017). DESs are classified into five distinct types (Table 13) (Smith et al., 2014).

Table 13. General classification of DESs

Type	General Formula	Terms
I—HBA and metal chloride	$Cat^+ X^- z MCl_n$	M = Zn, Sn, Fe, Al, Ga, In
II—HBA and metal chloride hydrate	$Cat^+ X^- z MCl_n y H_2O$	M = Cr, Co, Cu, Ni, Fe
III—HBA and HBD	$Cat^+ X^- z RZ$	Z = OH, COOH, $CONH_2$
IV—Metal chloride and HBD	$MCl_n + RZ = MCl_{n-1}{}^+ \cdot RZ + MCl_{n+1}$	M = Al, Zn; Z = OH, $CONH_2$,
V—Molecular compounds	Non-ionic DESs	Molecular substances

Cat^+: ammonium, phosphonium, or sulfonium cation. X^-: Lewis base (generally, a halide). HBA: hydrogen bond acceptor. HBD: hydrogen bond donor. R: alkyl rest. z: stoichiometric coefficient.

Most of the research has focused on Type III: DESs consisting of a quaternary ammonium salt as HBA (usually choline chloride) and a suitable hydrogen bond donor such as alcohols, carboxylic acids, or amides. This is because type III shows a stronger hydrogen bond interaction, which directly affects the physicochemical properties of DESs (Moldes *et al.*, 2022).

DESs starting materials are cheap, easy to produce, and do not entail associated pollution (most are allowed for human consumption). DESs are also more biodegradable than ILs (>69.3% in 28 days). DESs are applied in many fields such as electrochemistry, and organic and inorganic chemistry. In addition, DESs are being used in separation strategies replacing the role of ILs, as they are considered a greener alternative (Moldes et al., 2022). In addition, DESs are being used in separation strategies replacing the role of ILs, as they are considered a greener alternative.

According to the literature, DESs should also be critically reevaluated, as they encompass some downsides such as hygroscopicity, toxicity, renewability, and decomposition at high temperatures. To circumvent some of these problems, a DES-like liquid could be formed when mixing natural primary cellular metabolites such as choline, sugars, betaine, alcohols, organic acids, and amino acids (Moldes *et al.*, 2022). Thus, using natural products to prepare DESs (natural deep eutectic solvents or NADESs) will ensure low or negligible environmental impact and toxicity, as these new solvents are biodegraded more than 70% in 7 days, along with ubiquity and low cost (Moldes *et al.*, 2022).

NADESs are more eco-friendly and exhibit lower toxicity than DESs, which in turn are better in these and other aspects such as biodegradability, cost, and synthesis facility than ILs. NADESs have proved to be an ideal extraction media since many macromolecules such as DNA, proteins, carbohydrates, and other small molecules are highly soluble in some of these solvents such as 1,2-propanediol–choline chloride–water, glucose–choline chloride–water, or lactic acid–glucose–water (Dai *et al*., 2013).

Even though NADESs show numerous advantages and are considered capable of dissolving all sorts of biomolecules in living things, they also present some limitations, such as their high viscosity, which may prevent further industrial application. Nonetheless, this issue can be solved by increasing the operation temperature, or by adding water, which is preferred to maintain the structural activity of the proteins extracted (Mehariya *et al*., 2021).

Accelerated solvent extraction (ASE)

ASE is a sample preparation technique that is beneficial to several extracting processes in laboratories interested in the development of added-value products with applications in the food and feed industry, natural products, health care, etc. The accelerated solvent extraction technique uses elevated temperature and pressure to improve extraction efficiency and reduce extraction times to minutes per sample. Laboratories extraction processes will benefit from ASE due to several key advantages over ultrasonication, shaking, and stirring including true walk-away automation and selective extractions. The Accelerated Solvent Extraction technique is an exhaustive extraction technique that ensures high recovery of target analytes in complex plant matrices and reduces the productivity bottleneck caused by manual sample preparation techniques (Thermo Fisher Scientific, 2016).

ASE also known as Pressurized Liquid Extraction (PLE), is considered a green technique, which can be efficiently used to recover high-added-value compounds from food matrices and side streams. As an example, ASE is used to obtain aqueous protein extracts with in vitro antioxidant capacity from fish (rainbow trout, sole, sea bass, sea bream and salmon) side streams (De Aguiar Saldanha Pinheiro *et al.,* 2023). Ultimately, this technique supplies a cleaner sample for separation and detection of sample components during downstream chromatographic analysis, providing more accurate results.

When extractions occur at temperatures ranging from 50–200°C, several factors contribute to improved speed and efficiency (Table 14) (Thermo Fisher Scientific, 2016).

Table 14. Parameters that improve extraction efficiency

Parameter	Effect on the Extraction Process
Temperature	Elevated temperature increases analyte diffusion from the matrix and improves analyte solubility in the extraction solvent (e.g. methanol).
Analyte Solubility	Increases as temperature increases to improve extraction efficiency (e.g. solubility of anthracene increases 13-fold in DCM (50–150 °C)
Solvent Viscosity	Decreases as temperature increases. Improves solvent migration through the matrix to increase extraction efficiency
Solvent Surface Tension	Decreases as temperature increases. Allows solvent to better coat the matrix and helps improve analyte diffusion.

As an example, Figure 13 shows the results of the Accelerated Solvent Extraction technique vs. several other extraction techniques such as ultrasonication, stirring, shaking, and reflux. In this case, chlorogenic acid was extracted from eggplant samples using eight different techniques and the extracts were analysed using HPLC. The Dionex ASE system produced the highest recovery of chlorogenic acid and thus demonstrated greater extraction efficiency than manual extraction techniques (Thermo Fisher Scientific, 2016).

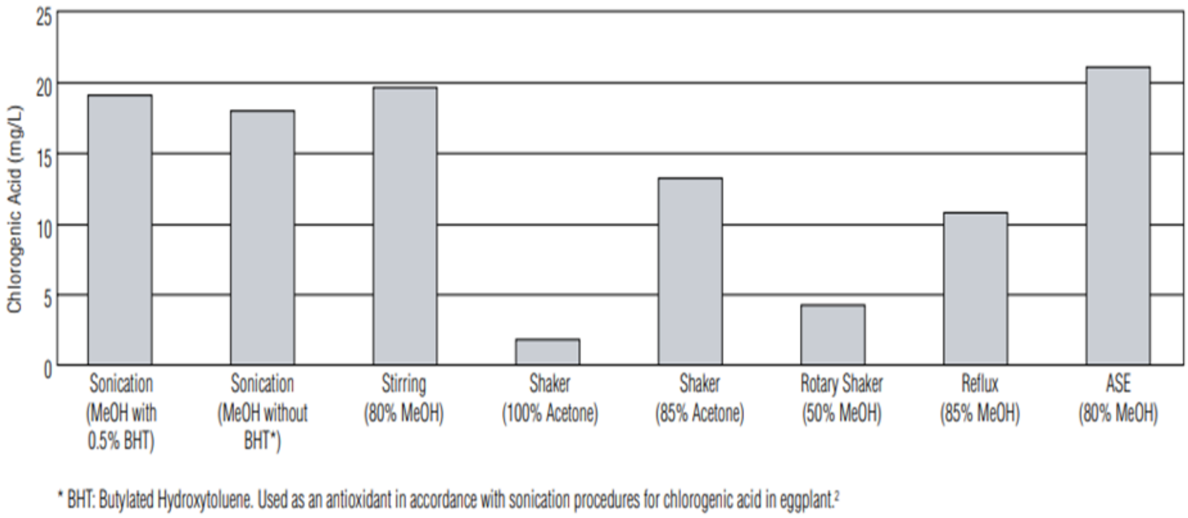


Figure 13. Comparison of extraction of chlorogenic acid from eggplant using eight different extraction methods

ASE is accepted in US EPA Method 3454A for extracting PAHs and semivolatile compounds, PCBs, dioxins, furans, TPH and explosive compounds as well as in CLP OLM 04.2A for semivolatiles and pesticides. This technique can be used to analyse food products for pesticide residues, lipids after acid hydrolysis, fat and additive content, and flavour profiles. Instruments can be used to automate the ASE process, mitigating variability inherent in manual sample preparation, and ensuring reproducible results. Automation makes ASE a fast, safe, and easy process. Where traditional techniques like Soxhlet and sonication take 8-10 hours and hundreds of millilitres of solvent, automated ASE requires just 15-30 minutes and 10-30 mL of solvent, depending on the application, and allows samples to be extracted overnight.

Accelerated solvent extraction applications.

Table 15 shows the common applications of Accelerated solvent extraction (ASE). The ASE technique is widely used and applicable to several economic sectors, making food and feed one of the most important.

Table 15. Common applications of ASE

Environmental samples	Pharmaceutical samples	Polymer samples	Food samples	Consumer products
Pesticides/herbicides PAHs/semivolatile PCBs Dioxins/furans TPH (DRO) Explosives Air sampling cartridges Brominated flame retardants	Active ingredients Drug delivery devices/packaging	Oils/organic acids Xylene solubles Polymer additives	Vitamins/antibiotics Contaminants Natural products Pesticides/herbicides Pesticide residues Fats/lipids Herbal/dietary supplements	Detergents Paper and pulp Biofuels Textiles and fibers

Source: Thermo Fisher Scientific, 2023.

3.3.3. Valorisation of residual biomass

3.3.3.1. Chitin and chitosan

The growing need to reduce dependence on fossil fuels has spurred interest in developing renewable materials for various applications Chitin, the second most abundant nitrogenous natural polymer sourced from renewable biomass, is gaining attention as a promising natural material for functional products due to its unique properties, widespread availability, and eco-friendly characteristics, particularly in sectors such as pharmaceuticals, food production, and waste management and remediation (Chakravarty & Edwards, 2022). Chitin and its derivatives, including chitosan, which is derived from deacetylated chitin, are polymers that evidence biocompatibility and biodegradability. The distinctive attributes of chitin, particularly its facile chemical modification, render it a compelling choice for the development of functional materials (Achinivnu *et al*., 2022).

Despite being present in substantial quantities in seafood waste, which is currently underutilized and leads to resource wastage, there is potential to upcycle chitin-containing waste into valuable goods. This approach aligns with sustainable development goals and could offer both ecological and economic benefits by tapping into the large quantities of seafood waste that are currently underexploited (Amiri *et al*.,2022).

Chitin, as found in nature, exists within a complex matrix of proteins, pigments, and calcium carbonate. Extracting chitin involves multiple steps, including demineralization, deproteination, and pigment removal. In this process, two significant constituents of shells, proteins, and inorganic calcium carbonate ($CaCO_3$), must be eliminated. Traditional chitin extraction methods typically consist of two fundamental steps: (1) deproteinization to remove proteins and (2) demineralization to remove minerals (Kulawik *et al*., 2019). A newer industry addition involves the decolorization of chitin to eliminate pigments, resulting in a colourless and pure chitin. Various methods, including chemical and biological processes, have been employed to produce chitin, with specific steps depending on the source of chitin extraction (Chakravarty & Edwards, 2022).

Chemical extraction

The extraction of pure chitin on a commercial scale involves harsh treatments due to its complex organization with other constituents. Chemical demineralization is a crucial step that uses acids such as HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH to remove mineral constituents, primarily calcium carbonate and calcium phosphate, from crustacean shell waste. Demineralization, typically lasting 2 to 3 hours, varies based on factors like shell source, size, extraction temperature, acid concentration, and solute/solvent ratio. However, the high temperature and extended process time during demineralization can negatively impact the properties of the chitin polymer (Kaur & Dhillon, 2015).

Chemical deproteinization, involving an alkali treatment, follows demineralization. This step employs substances such as NaOH, Na_2CO_3, Na_3PO_4, Na_2S, KOH, and K_2CO_3 to remove proteins from demineralized shells. The deproteinization step is acknowledged to be challenging, potentially due to the disruption of covalent bonds between chitin and proteins (Kaur & Dhillon, 2013).

The critical factors in chitin synthesis are the quality of the final product, gauged by parameters such as molecular weight, polydispersity, and the degree of acetylation. The use of harsh acid treatments in the demineralization stage can lead to hydrolysis of chitin, resulting in inconsistent physical properties, which adversely impact molecular mass and acetylation degree. These issues not only affect the intrinsic properties of purified chitin but also contribute to environmental pollution. Additionally, high NaOH concentrations and elevated temperatures during deproteinization steps may cause undesired deacetylation and depolymerization of chitin, thereby limiting its suitability for various applications (Kaur & Dhillon, 2013).

Chitin extraction through chemical processes is highly hazardous, energy-intensive, and contributes to environmental pollution. The associated chemical treatments pose disposal challenges, requiring wastewater neutralization and decontamination to comply with strict government regulations. Additionally, the valuable protein components are damaged during chemical extraction, rendering them unsuitable as animal feed. Due to the significant impact on chitin quality and the environment, there is a growing interest in biological techniques for chitin extraction as an alternative approach (Chakravarty & Edwards, 2022).

Biological extraction

Biological methods for chitin extraction involving diverse microorganisms are considered simpler, more productive, and environmentally friendly compared to chemical extraction methods. These biological approaches preserve the chitin structure better than chemical methods. Fermentation processes, particularly those using proteolytic microorganisms, have been extensively studied for chitin extraction from various sources such as shrimp shells, lobster shells, prawn waste, and crab shells (Ahuja *et al*., 2021). Different fermentation procedures, including single-step and two-step fermentation, mono- and co-fermentation, have been explored. Co-culture utilization is noted to be advantageous, potentially offering synergistic benefits by harnessing the metabolic pathways of all involved strains in a co-culture condition (Chandrasekharan, 2015).

Production process emissions

Conventional techniques for chitin extraction may pose environmental issues, such as emissions of pollutants or the use of harsh chemicals. In the demineralization and deproteinization phase, dilute hydrochloric acid (HCl) is utilized, while a dilute sodium hydroxide (NaOH) solution is employed for protein removal. Additionally, during the deacetylation step, highly concentrated (40–50%) solutions of NaOH are applied at elevated temperatures. The production and utilization of these chemicals, particularly hydrochloric acid, and sodium hydroxide, can give rise to emissions, especially if their handling and disposal are not appropriately managed. Developing cleaner and more sustainable production processes is crucial (Muñoz *et al*., 2018).

Energy-intensive

The energy-intensive aspects of certain chitin extraction processes contribute significantly to emissions and the carbon footprint of the valorisation process. High temperatures and prolonged reaction times are key factors in this environmental impact.

Specifically, the traditional method for chitin and chitosan production, particularly the deproteinization step using marine waste material, involves the application of strong acids and bases at high temperatures (100-110°C). This approach demands substantial energy inputs and results in effluents that require neutralization through appropriate treatments (Percot *et al*., 2003).

In addition, the production of enzymes and chemical reagents, even in small quantities, may counterintuitively demand more energy and raw materials than it saves (Nielsen *et al*., 2007; Kim *et al*., 2009). This points to the nuanced environmental considerations associated with these processes.

The impact on enzyme consumption in enzymatic production is noteworthy, attributed to both the low natural productivity of the raw material used and the energy-intensive nature of the process (Lopez *et al*., 2018).

Energy usage is a pivotal factor, with the utilization of coal as a fuel during chitin production and overall electricity consumption significantly influencing climate change, acidification, and ecotoxicity impacts (Muñoz *et al*., 2018). Therefore, addressing the energy-intensive aspects of chitin extraction processes is crucial for mitigating their environmental footprint.

Waste management

While the valorisation of chitin contributes to the reduction of biomass waste, it is essential to address potential environmental concerns arising from the processes involved. Traditional methods may give rise to by-products or waste that demands meticulous management to prevent adverse environmental effects.

Conventional chitin extraction processes generate various waste streams, including wastewater and Waste NaOH. Wastewater produced during chitin and chitosan production undergoes on-site treatment, involving neutralization, primary settling, biological treatment, and sand filtration. This ensures that the discharged water meets environmental standards. For example, to manage this, on-site treatment is implemented before discharging treated effluents into the sea. The extracted protein, in sludge form, is locally recycled as a fertilizer, while calcium salts find use in landfills or as road-filling material (Muñoz *et al*., 2018).

However, chemical treatments in the chitin extraction process pose additional challenges. The resulting wastewater may require naturalization and decontamination to comply with stringent government regulations. Improper disposal of waste, including by-products and unused biomass parts, could contribute to emissions, emphasizing the importance of effective waste management practices in the chitin extraction industry.

The valorisation of chitin and chitosan from biomass presents an environmentally promising avenue, but it requires a holistic approach that addresses both the potential benefits and challenges associated with their production, use, and disposal. Sustainable practices, green technologies, and a focus on minimizing environmental footprints are essential for realizing the full environmental potential of chitin and chitosan valorisation.

Strategies to improve.

- Green Chemistry Practices: Adopting green chemistry principles can minimize the use of hazardous chemicals and reduce emissions associated with the production process.
- Energy Efficiency: Implementing energy-efficient technologies and optimizing process conditions can help minimize the carbon footprint of chitin valorization.
- Waste Valorization: Finding ways to utilize by-products and waste generated during chitin extraction can contribute to a more sustainable process, reducing emissions associated with waste disposal.
- Sustainable Sourcing: Prioritizing sustainable and responsibly sourced biomass, such as waste from seafood processing, can reduce the overall environmental impact.
- Circular Economy: Incorporating principles of the circular economy, such as recycling and reusing chitin materials, can minimize the need for new production and associated emissions.

3.3.3.2. Fermentation medium

A fermentation medium, often simply referred to as a "medium," is a nutrient-rich substance or mixture of substances used to support the growth and metabolic activity of microorganisms during the process of fermentation. Fermentation is a metabolic process wherein microorganisms, such as bacteria, yeast, or fungi, convert sugars or other organic compounds into various products, such as acids, gases, or alcohols.

The fermentation medium serves as the environment in which the microorganisms thrive, reproduce, and carry out the desired metabolic activities. It provides essential nutrients, including carbon and nitrogen sources, minerals, vitamins, and other components necessary for microorganisms to grow and produce the desired fermentation products.

The composition of a fermentation medium can be tailored to the specific requirements of the microorganism and the desired end product. The medium's formulation is a critical factor in optimizing the efficiency of the fermentation process, ensuring high yields of the desired product, and controlling the overall production costs.

SCP production through fermentation involves a biochemical process executed by microorganisms like yeast, bacteria, and fungi. These microorganisms break down intricate substrates into simpler compounds to support their growth (Nasseri *et al*., 2011). The feedstock and waste utilized in SCP production primarily come from agricultural sources. The resulting product can serve as a protein supplement in both food and feed applications.

Anupama and Ravindra (2000) propose that fermented products containing Single Cell Protein (SCP) can serve as a cost-effective alternative to traditional protein sources like fishmeal and soymeal for both human and animal consumption. It is emphasized that microorganisms considered "generally regarded as safe (GRAS)" should be employed for fermentation. Additionally, its immunostimulating compounds make it suitable for applications like aquafeed production through fish waste fermentation, where lemon peel is used as a natural filler to reduce liquefaction during the process (Tropea *et al*., 2022).

In the production, the selected strain undergoes a fermentation process, utilizing a substrate as a carbon source. The resulting culture mass is separated through a specific isolation process. The cultivation process begins with microbial screening, obtaining strains from soil, air, and water, and determining all pathways and cultivation conditions. Attention is given to environmental protection and safety considerations concerning both the process and the yield. Various types of fermentation are distinguished as follows (Abdur *et al*., 2020):

Submerged fermentation

- In this process, the substrate remains in liquid form, providing essential nutrients for microbial growth.
- The fermenter continuously operates the substrate, and the product is regularly harvested through techniques such as centrifugation or filtration.
- Aeration is crucial to remove heat generated during the process, facilitated by a cooling device.
- Microbial biomass, including single-cell proteins like filamentous fungi, is separated using methods such as filtration, while yeast and bacteria are separated through centrifugation.

Semisolid fermentation

- In semisolid fermentation, the substrate is predominantly in a solid state.
- The cultivation process involves operations like stirring, mixing of multiphase systems, and the transport of oxygen, with heat removal to the surroundings.

- The U-loop fermenter is a specialized bioreactor designed for identifying mass and heat transportation.
- Key steps in preparing the medium for SCP production include selecting a suitable medium and carbon source, preventing medium contamination, and isolating the end product.
- Carbon sources may include methanol, ethanol, gaseous hydrocarbons, n-alkanes, carbon oxide, polysaccharides, or molasses.

Solid state fermentation

- Involves depositing a solid substrate (e.g., wheat bran or rice) on flatbeds after seeding with microbes and allowing it to sit in a temperature-controlled room for a few days.

The production of SCP through fermentation can have several environmental considerations and challenges. Here are some of the environmental issues associated with the fermentation medium used for SCP production:

Waste generation

The production of SCP involves the cultivation of microorganisms on a nutrient-rich medium, and waste generation can occur at various stages of the process. Here are some key points related to waste generation in the fermentation medium for SCP (Singh *et al.*, 2017):

- Unused Substrates: In the fermentation process, not all of the substrate (carbon source) may be utilized by the microorganisms. Unconsumed substrate can be considered waste and may need proper disposal or treatment.
- Metabolite By-Products: Microorganisms produce metabolic by-products during fermentation. These by-products may include organic acids, gases, and other compounds. Depending on the specific fermentation process, these by-products may need to be managed to prevent environmental impacts.
- Biomass Residues: After the fermentation is complete, there is often a residue of microbial biomass that may not be used for the intended product. This biomass can be considered waste and may need proper disposal or further processing.
- Filtration and Separation Residues: Filtration and separation processes are used to isolate the culture mass or extract the desired product. Residues from these processes, such as filter media and solid particles, may require appropriate handling and disposal.

- Unused Nutrients and Additives: Nutrients, vitamins, and other additives added to the fermentation medium may not be fully consumed during the process. The leftover unused additives may contribute to waste.

Energy consumption

The fermentation process often requires energy for aeration, agitation, temperature control, and other operational aspects. Depending on the energy sources used, this can contribute to environmental concerns, especially if the energy is derived from non-renewable resources. Here are some key factors related to energy consumption (Curran *et al*., 1989):

- Aeration and agitation: Aeration and agitation are crucial for providing oxygen to the microorganisms and maintaining a homogenous culture in the fermentation medium. These processes often require energy, especially in large-scale industrial fermentations.
- Temperature control: Maintaining optimal temperature conditions for microbial growth is essential. Heating or cooling the fermentation medium to the desired temperature consumes energy. Energy-efficient temperature control methods are critical for minimizing overall energy consumption.
- Mixing and stirring: Ensuring uniform distribution of nutrients and maintaining a consistent environment throughout the fermentation process requires mixing and stirring. The energy used in these mechanical processes contributes to overall energy consumption.
- Downstream Processing: Energy is also consumed during downstream processing steps, such as separating the microbial biomass from the fermentation medium, concentrating the product, and other purification processes.

Raw material source

The cultivation medium's components, such as carbon and nitrogen sources, may be derived from agricultural or industrial processes. Unsustainable sourcing of these raw materials can lead to environmental degradation, deforestation, or depletion of natural resources (Singh *et al*., 2017).

- Carbon Source: Carbon stands out as the pivotal component within the medium, serving as the primary energy source for microorganisms. It holds a significant role in both growth and the generation of primary and secondary metabolites. The pace at which the carbon source undergoes metabolism frequently affects the formation of biomass and the production of primary or secondary metabolites. Common carbon sources include sugars (glucose, sucrose), starch, or industrial

by-products like molasses. Sourcing sustainable and cost-effective carbon substrates is essential for the economic feasibility of SCP production.
- Nitrogen Source: Nitrogen is a critical component for protein synthesis. Like carbon, the choice of nitrogen source and its concentration in the media is equally vital for metabolite production. Microorganisms have the capability to utilize both inorganic and/or organic nitrogen sources. Sources such as ammonium salts, urea, or organic nitrogen sources like yeast extract or peptone are used. Ensuring a stable and reliable nitrogen supply is crucial for consistent SCP production.
- Phosphate: Phosphate serves as an essential component necessary for synthesizing phospholipids found in microbial cell membranes and to produce nucleic acids. The quantity of phosphate added to the fermentation medium is contingent on the composition of the broth, the organism's requirements, and the specific nature of the desired product. Sourcing this component responsibly is essential to prevent environmental degradation.

The description provided highlights those alterations in the carbon or nitrogen sources within the production medium or deviations from their optimal concentrations, that can impact the characteristics of the product and its overall productivity. Hence, a production medium containing all necessary components in suitable concentrations is essential for large-scale production of the desired metabolite. To standardize the production medium, the concept of medium optimization has been introduced.

Water use

As Stanbury *et al*. (2017) mentioned in their book, fermentation processes typically require significant amounts of water. Water constitutes a significant portion of nearly all fermentation media and plays a crucial role in various auxiliary functions such as heating, cooling, cleaning, and rinsing. A substantial quantity of consistently clean water is essential and should be sourced from reliable and permanent outlets. When evaluating the appropriateness of a water supply, factors such as pH, dissolved salts, and the potential for effluent contamination should be carefully considered. The responsible use of water resources is essential to prevent water scarcity and minimize the environmental impact associated with excessive water consumption.

- Initial Medium Preparation: Water is a fundamental component in the preparation of the fermentation medium. The quality and quantity of water used at the beginning of the process significantly impact the overall fermentation performance.
- Nutrient Dissolution: Water is required to dissolve and distribute nutrients, such as carbon and nitrogen sources, minerals, and other additives, evenly throughout

the fermentation medium. This step is critical for providing a suitable environment for microbial growth.

- Aeration and Oxygen Supply: Water is often used as a carrier for providing oxygen to the microorganisms during aeration. Efficient oxygen supply is crucial for the growth and metabolic activities of the microorganisms in the fermentation process.
- Temperature Control: Water is used for maintaining optimal temperature conditions during fermentation. Cooling systems may utilize water to dissipate heat generated during microbial activities.
- Product Harvesting and Separation: Water may be involved in downstream processes such as harvesting and separating the microbial biomass or extracting the SCP product from the fermentation medium.

Strategies to improve

Addressing these environmental issues often involves adopting sustainable practices, optimizing resource efficiency, implementing waste reduction strategies, and using eco-friendly alternatives in the fermentation medium. Continuous research and innovation in SCP production processes aim to minimize their environmental footprint.

Mitigating the environmental impact of waste generation in SCP production involves the adoption of sustainable practices and waste reduction strategies. These strategies encompass optimizing fermentation conditions, enhancing substrate utilization efficiency, and implementing efficient downstream processing methods to minimize residual waste. Additionally, a focus on environmentally friendly disposal or recycling methods is crucial to address any waste generated during the fermentation process.

Efforts to reduce energy consumption in SCP fermentation processes revolve around strategic approaches as process optimization, where fermentation conditions such as temperature, pH, and nutrient concentrations are fine-tuned to enhance efficiency and diminish the need for excessive energy inputs. Bioreactor design plays a significant role, with the development and utilization of bioreactors featuring improved designs that enhance mixing, aeration, and heat transfer efficiency, thereby reducing overall energy requirements. Further strategies involve exploring alternative energy sources like solar or wind energy to power fermentation processes and incorporating waste heat recovery systems to reuse heat generated during fermentation for other aspects of the process or facility (Yaroshevsky, 2019).

Considerations for sustainable raw material sourcing in SCP fermentation are multifaceted. Sustainability practices involve consciously choosing raw materials from environmentally friendly sources to minimize the ecological impact of SCP production.

The exploration of waste by-products from other industries as raw materials promotes a circular economy, effectively reducing the overall environmental impact. Local sourcing is encouraged to minimize transportation-related environmental effects and to support local economies. Additionally, the exploration of biorefinery concepts, where multiple products can be derived from the same raw material, enhances overall resource utilization efficiency (Singh *et al.*, 2017).

Efforts to improve water use efficiency in SCP fermentation involve several key strategies. Recycling and reuse systems are implemented within the fermentation process to minimize overall water consumption. The use of treated or purified water helps reduce the risk of contamination and ensures optimal fermentation conditions. Process optimization is applied to reduce the need for excess water, especially during aeration and cooling processes. Sustainable water sourcing practices are considered to minimize the environmental impact associated with water extraction. Finally, closed-loop systems are implemented, allowing water to be circulated and reused within the fermentation process, thus reducing the demand for fresh water (Stanbury *et al.*, 2017).

3.3.3.3. Bioplastics and biostimulants

The modernization of the world and the necessity for prosperous societies have increased the standards of living and thus food safety and quality levels. Predictions for global population growth of up to 9.7 billion by 2050 will inevitably lead to increased competition for land, water, and energy. Sustainable food systems based on renewable food ingredients are vital to be developed. As a result, issues related to greenhouse gas emissions (GHG), increased usage of fresh water (approx. 70%) for agriculture and GHG emissions related to livestock production (up to 18%) as well as ammonia utilization could be overcome or at least deteriorated (Koukoumaki *et al.*, 2023).

Alternative protein sources such as plant by-products, insects, and microorganisms have attracted scientific attention since they do not demand arable land and they can be produced utilizing renewable feedstock (waste and/or by-product streams) derived from numerous agri-food residues. Among these non-conventional sources, microorganisms present enhanced protein content. Dry cell biomass has been described as 'single cell protein' (SCP) or 'microbial protein'. SCP is primarily sourced from microorganisms, and it constitutes an eco-friendly substitute for animal-derived proteins (Figure 14). The global demand for proteins is continuously growing while advances in the food processing sector are likely to trigger SCP importance, although the latter still holds a small market share. More specifically, the market of SCP is forecast to exceed $18.5 billion by 2030 (Koukoumaki *et al.*, 2023).

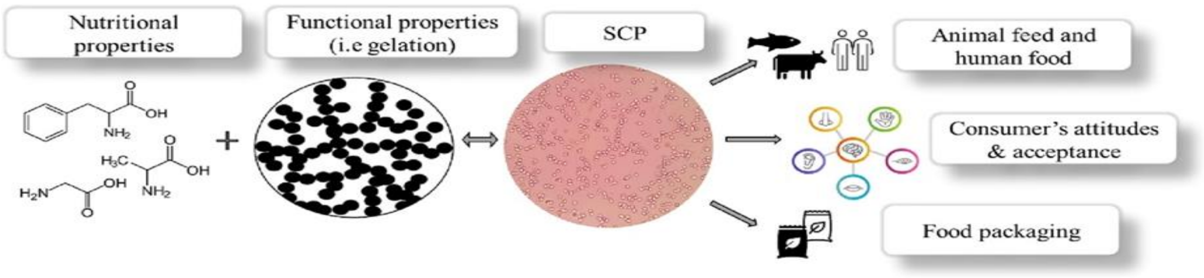


Figure 14. Properties and applications of SCP

SCP can be involved in a plethora of applications including both the agricultural and commercial sectors. More specifically, SCP has been used as a protein source for fish meals, foam-stabilising agent, in paper and leather processing, as a potential packaging material and as a good candidate for animal feed supplements due to its nutritional value as well as due to the potential influence in the reduction of enteric CH_4 emissions in ruminant animals (Figure 15) (Koukoumaki *et al*., 2023).

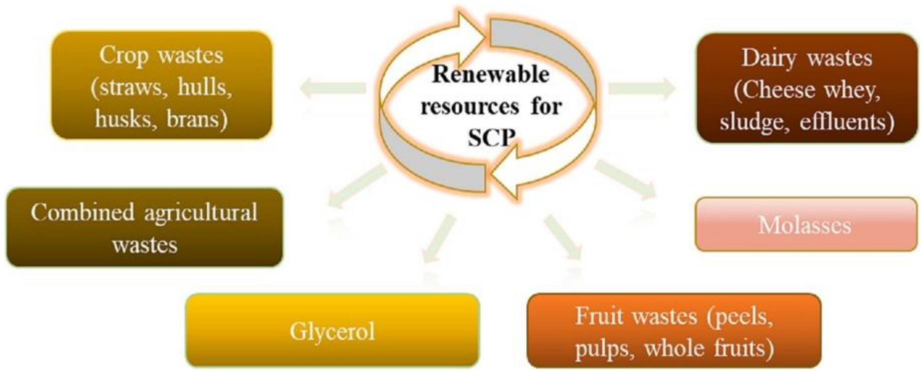


Figure 15. Renewable Resources for SCP production

New developments require innovative eco-friendly materials defined by their biocompatibility, biodegradability, and versatility. For that reason, scientific society is focused on biopolymers such as chitosan, which is the second most abundant in the world after cellulose. These new materials should show good properties in terms of sustainability, circularity, and energy consumption during industrial applications (Maliki *et. al*., 2022).

The new regulations promoted by numerous governments are trying to take care of the environment by protecting actions and behaviours to develop a new sustainable economy. Some of the most important goals of these laws are aimed at the reduction of the excessive consumption of non-renewable raw materials, especially those derived from natural sources. The extraction and cleaning of raw materials are responsible for soil degradation, biodiversity loss, water shortages, and global warming. The use of residues as raw materials is a new concept derived from the circular economy which could contribute to the reduction of the huge amounts of trash accumulated in landfills. The concept of a circular material means that a new product can be obtained from the old one which acts as a raw material (Maliki *et al*., 2022).

Chitosan is used in a wide range of applications and industries, related to agriculture, pharmacy, medicine, food, or textile among others, but also in new developments such as biomedicine, biotechnology, wastewater treatment, catalysis, packaging, or bioimaging which are essential for a new sustainable era where chitosan can provide versatility, recyclability, and low cost. Chitosan is obtained through the deacetylation of chitin, which is one of the most abundant biomaterials after cellulose. This one is a polysaccharide which can be found in crustaceans, insects, or fungi (Hamed *et al*., 2016). Chitin is considered a linear long-chain homopolymer which is composed of N-acetyl glucosamine and can develop three polymorphic forms known as α-, β-, and γ-chitin (Table 16).

Table 16. Main chitin sources and percentages

Source	Percentage (%)
Shrimps	30–40%
Squids	20–40%
Krill	20–30%
Crabs	15–30%
Fungi	10–25%
Insects	5–25%
Oysters	3–6%
Clams	3–6%

The extraction of chitin is necessary to produce chitosan as previously explained. A huge amount of chitin is obtained from crustaceans, but there are multiple advances in its production through insects or fungi and bacteria, thus avoiding the use of animal derivatives. In general, extraction requires several steps starting with the removal of mineral salts and proteins (Figure 16) (Maliki *et al*., 2022).

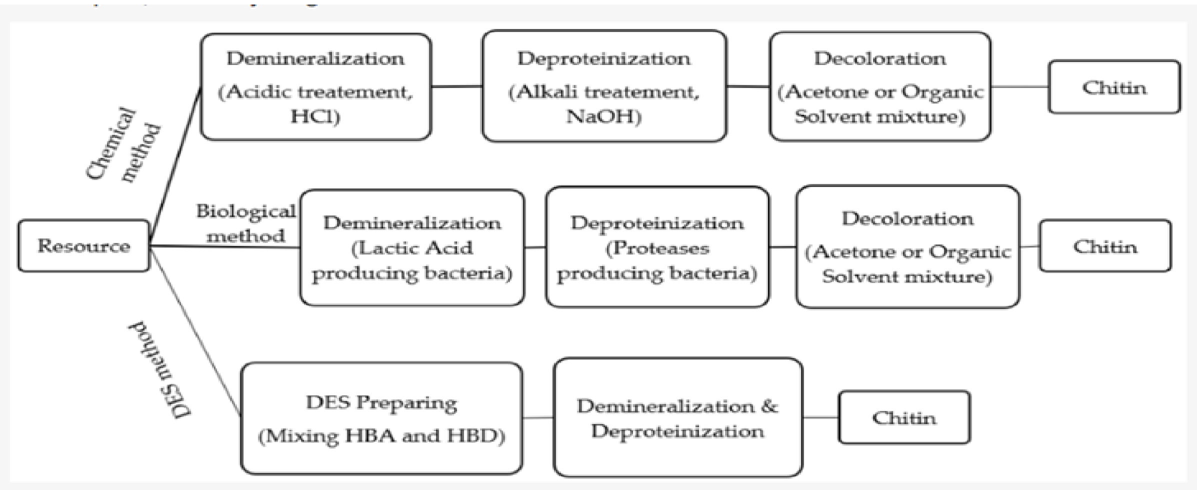


Figure 16. Extraction of chitin. DES: deep eutectic solvents; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor

Increasing concern regarding the environmental impact of plastic waste and the plastic-related emission of greenhouse gases (GHGs) motivates the transition towards a 'circular plastic economy'. In a circular economy, the use of non-renewable resources and waste production is minimized, while reuse and recycling dominate the life cycles of materials (Rosenboom *et al*., 2022).

Although most commercial plastics are made from fossil resources, these materials can also be made from renewable resources and are commonly referred to as bioplastics. Biomass extraction can also yield non-synthetic natural polymers, such as starch, natural rubber, and proteins. Bioplastics are plastics that are either made from renewable resources ('bio-based'), biodegradable or are made through biological processes or a combination of these. Some biodegradable but fossil-based plastics are also referred to as bioplastics; however, the use of this terminology is advised against, as it is misleading (Rosenboom *et al*., 2022).

Bioplastics that are 100% bio-based are currently produced at a scale of approx. 2 million tonnes per year and are considered a part of future circular economies to help achieve some of the United Nations (UN) Sustainable Development Goals (SDGs), such as by diverting from fossil resources, introducing new recycling or degradation pathways, and using fewer toxic reagents and solvents in production processes. Depending on the type, bioplastics can offer improved circularity by using renewable (non-fossil) resources, a lower carbon footprint, biodegradation as an alternative end-of-life (EOL) option and improved material properties. These benefits, however, are highly dependent on several factors, including the chemical structure, the manufacturing process and the most likely EOL scenario. All these factors must be evaluated across the life cycle, along with metrics such as climate impact, ecotoxicity and recyclability, using tools such as a life cycle assessment (LCA) to elucidate the environmental benefit over alternatives (Walker &. Rothman, 2020).

Climate change

Plastic production consumes between 5–7% of the global oil supply and releases more than 850 million tonnes of CO_2 into the atmosphere in 2019, representing 2% of the global CO_2 output. Most plastic-related CO_2 emissions are incurred by raw material extraction (61%) and polymer production (30%), while only 9% is associated with the End-Of-Life (EOL) stage, mainly incineration. Simulations suggest that complete replacement of fossil feedstocks with sugarcane would reduce GHG emissions by approximately 25% but recycling all existing plastics could have a similar effect. As the largest carbon footprint of plastics is associated with production, switching existing processes to a renewable energy supply would cut plastic-related emissions by 62% (Rosenboom *et al*., 2022).

End-of-life and circular economy principles

In an ideal circular economy, plastics would be made from renewable or recycled resources. However, the traditional life of most plastic materials is linear. 79% of all plastic produced has been dumped into landfill sites or reached the environment, while the remainder has been incinerated (12%) or recycled (9%). Although recycling has increased since the 1980s, the recycling of non-fibre plastic has stagnated at 18% and almost no textile fibres are recycled. The lack of progress is largely due to the limitations of the dominant form of recycling — mechanical recycling — which converts waste plastics into new shapes through mechanical force and heat. Product quality is, therefore, highly dependent on input quality and, thus, mechanical recycling ideally requires well-sorted and contamination-free plastic waste, which is often scarce (Rosenboom *et al*., 2022).

By contrast, different forms of chemical recycling offer a more resilient and flexible way to recycle mixed and contaminated plastic waste, as well as popular multilayer materials. Chemical recycling first depolymerizes the polymer to recover the monomers, which, after appropriate separation, can undergo repolymerisation into materials of defined quality. However, these processes are more complex and, thus, more expensive, particularly during the implementation phase, and, therefore, require financial incentives (Rosenboom *et al*., 2022).

Biodegradation is an EOL option largely for easily hydrolysable polymers, such as aliphatic esters like polylactic acid (PLA) but should be performed only in controlled industrial settings to ensure complete digestion without uncontrolled side effects, such as leakage of contaminants or microplastic formation (Rosenboom *et al.,* 2022).

Innovating 'beginning-of-life' options to minimize environmental impact is equally important as EOL considerations. Educating consumers and companies towards 'life cycle thinking' will encourage a holistic view of plastic products beyond their obvious impacts associated with the use and disposal of plastics. Less tangible but potentially more detrimental environmental impacts are associated with feedstock harvesting, processing energies and transportation. To this end, the sustainable harvesting and catalytic conversion of local, non-food, renewable resources and biological wastes into bio-based plastics can provide greater sustainability than established fossil fuel extraction and refining practices (Rosenboom *et al*., 2022).

Circular economy principles and technologies, therefore, need to be enacted at every step along the plastic supply chain to minimize environmental impact and plastic waste. Useful measures towards future circular economies include a drastic reduction in plastic consumption, the design of products that can be reused and recycled in their markets, improved process energy efficiency in plastic and bioplastic manufacturing combined with the use of renewable power, increased collection rates and market penetration of robust and circular recycling and 'upcycling' methods (Rosenboom et al., 2022).

Life cycle assessment of bioplastics

LCAs can be performed with various boundaries that result in different outcomes. Scenarios can be set up from cradle-to-gate (resource extraction to plastic factory outlet), cradle-to-grave (resource extraction, plastic manufacturing, and disposal process) and cradle-to-cradle (entire process including recycling or biodegradation processes at end of first life). There are international standards in place to guide the structure, conduct, limitations, and assumptions made of general LCAs, such as ISO 14040, and specific guidelines for bioplastics, such as EN 16760. However, heterogeneity in LCA approaches and assumptions persists. For example, the manufacturer's processing parameters are mostly confidential and, therefore, assumptions are typically made. Many LCAs focus on carbon emissions in cradle-to-gate scenarios, often for lack of reliable data on EOL scenarios (Rosenboom *et al*., 2022).

Bio-based plastics are not, by definition, more sustainable than fossil-derived plastics. In many LCAs, bioplastics yield a considerable reduction in global warming potential using renewable resources. However, these benefits are often counterbalanced by side effects of feedstock farming, such as acidification potential and eutrophication due to increased fertilizer and pesticide use. Improvements in the supply chain of raw material extraction, such as measures that eliminate extensive pesticide use and forest burning practices, could substantially reduce these negative impacts of bioplastics. LCA outcomes depend heavily on assumptions made for energy use and processing efficiencies in all production stages and, thus, some LCAs have associated bioplastics, such as PLA, with higher energy and water use. Bioplastics often scores negatively in landfill scenarios, whereas the situation looks more promising once plastic recycling is considered. Social and economic sustainability should also be included in LCAs to evaluate whether bioplastics contribute not only to a circular economy but a truly sustainable circular economy (Rosenboom *et al*., 2022).

3.4. Conclusion

Global food and feed demands are growing rapidly. Agriculture and livestock expansions show detrimental long-term implications for the environment. Within this framework, insect farming appears as an increasingly attractive candidate for a strategic response to the need for sustainable food and feed production. Insects represent an abundant food source that can be reared using organic side-streams, thus enhancing circularity and sustainability, while alleviating food and feed insecurity.

The utilization of microalgae biomass to produce SCP holds great promise as a sustainable and resource-efficient alternative. Microalgae's rapid growth, high protein content, and ability to thrive in various environmental conditions make them an attractive candidate for SCP production. The process offers numerous environmental benefits, including reduced land and water usage compared to traditional protein sources. Furthermore, microalgae cultivation has the potential to utilize non-arable land and wastewater, contributing to the concept of circular economy and resource recycling. However, challenges such as cost-effectiveness, scalability, and optimization of cultivation processes still need to be addressed. Continued research and technological advancements in microalgae cultivation, strain selection, and downstream processing will play a pivotal role in unlocking the full potential of microalgae biomass as a sustainable source of Single Cell Protein. As these challenges are overcome, microalgae-based SCP production could emerge as a crucial component in addressing global food security and promoting sustainable protein production practices.

At present, commercial insect rearing is growing in an industrial sector across North America and Europe. Nevertheless, the full potential of insects in terms of food and feed security can be realized, only if a formidable amount of work is done by a range of stakeholders in the next future. Contributions from academia and research institutes are fundamental to providing new insights into biological, chemical, and technological aspects concerning the production and use of insects as food and feed. Nevertheless, the direct and constant dialogue with the final users, such as consumers and farmers, must be improved to overcome both practical obstacles and scepticism. Moreover, public institutions and policymakers should ensure proper support to the industrial sector involved in insect farming, thus recognizing that it is an essential sector for developing more sustainable food chains.

To solve the upcoming protein shortage, new alternatives to animal protein must appear, reducing the environmental consequences of current protein production processes. Microalgae biorefinery is a bright answer to this problem because it is an excellent protein source (around half of the microalgae dry weight is protein, containing all nine essential amino acids). At the same time, these microorganisms can reduce atmospheric CO_2. However, the recovery of proteins from the microalgae matrix is complicated, and it is usually considered the bottleneck of the process.

Economic carbon compounds, such as agricultural waste and methanol have the highest potential in becoming widely used as carbon sources for SCP production. The key considerations for choosing the most suitable waste product and biomass for SCP production are:

- Local availability of the waste product (to reduce transportation costs);
- Pre-treatment costs of the waste product
- Maximum obtainable cell densities in substrate;
- SCP concentrations in the final biomass;
- Energy costs of cultivation conditions
- Efficiency of biomass and waste separation methods;
- SCP extraction (protein extraction from biomass and removal of impurities) methods

For protein recovery, multiple available techniques allow protein obtaining, and depending on their fundamentals, different purity levels will be attained. The solvent extraction method is one of the most employed, yet organic solvents go against the sustainability desirable for the process. In this context, ILs, and especially DESs, have been revealed as a greener alternative for protein extraction due to their excellent physicochemical properties that enable an efficient and environmentally friendly recovery.

Accelerated solvent extraction (ASE) is an innovative sample preparation technique that combines elevated temperatures and pressures with liquid solvents to achieve an efficient extraction process. With proper sample preparation and optimization of extraction parameters, nearly any sample currently extracted with a liquid solvent can be performed in less time and with smaller quantities of solvent using accelerated solvent extraction.

Tri-Phasic Partitioning (TPP) technology emerges as a promising and efficient approach for protein recovery across diverse applications. Its selective partitioning of proteins based on their characteristics provides advantages such as high purity and yield. The versatility of TPP is evident in its application across various industries, including pharmaceuticals, biotechnology, and food processing. Notably, TPP's environmentally friendly nature, avoiding the use of toxic solvents, enhances its appeal for sustainable protein recovery processes. However, ongoing research is crucial to optimize and scale up TPP for industrial use, addressing challenges related to cost-effectiveness and process scalability. As technology progresses and a deeper understanding of TPP is gained, it holds significant potential to revolutionize protein recovery, contributing to a more sustainable and efficient protein production landscape.

Bioplastics and biostimulants produced from chitin, chitosan, and proteins used as renewable resources alone do not imply sustainability. It is highly dependent on how a material is made, where it is used and how it can be recycled, and less so on the building blocks of a material. Nevertheless, with technological advances, bioplastics have the potential to move several plastic-intensive industries towards a circular economy. Bio-based replacements are available for almost every fossil-based application; however, these are mostly in small and costly quantities and do not always have substantial environmental benefits. Besides first-generation biomass, lignocellulosic agricultural and other biowastes present a renewable, abundant, and more ethically viable feedstock. However, biorefinery processes must increase efficiency and adhere to green chemistry principles (such as using non-toxic chemicals and reducing the energy demand) to supply polymer building blocks in a cost-competitive and sustainable manner.

The ability to evaluate, scrutinize and compare sustainability and the environmental impact of fossil-based and bio-based materials remains essential. LCA is the main tool but requires homogenization of methodology standards to make LCAs more transparent, consistent, and comparable.

The COVID-19 pandemic has highlighted that there is an urgent requirement for methods that can recycle increasing amounts of mixed waste streams of potentially poor quality, containing contaminants from the medical, food and other sectors. Innovation and financial incentivization in advanced recycling technologies, such as chemical and biological recycling, would further unlock (bio)plastic circularity. With improvements in efficiency and cost competitiveness, these techniques can enable the upcycling of plastic waste.

4. Task 1.4. Regulatory Requirements

4.1. Introduction

In order for InnoProtein proteins to achieve market approval within both the EU and international markets it is essential that these proteins are compliant with the relevant national, European, and international regulations and standards. With this in mind, all of the regulations and standards that have been complied in this document are in place to allow the InnoProtein project partners to achieve complete compliance for these proteins. The regulations and standards provided in this document cover the broader aspects of the InnoProtein project with regards to general food and feed regulations. However, there is a significant focus on regulations relating to specific work packages and tasks, as well as the individual project partners.

The purpose of Task 1.4. is to develop an inventory of national, European, and international regulations and standards that will affect the InnoProtein project, directly or indirectly. This inventory will be a reference guide for the InnoProtein partners, providing them with all of the relevant regulatory information they require to conduct their work throughout the project. It is intended that the regulations and standards be monitored closely, and this inventory updated regularly to ensure that the InnoProtein project remains complaint throughout.

4.2. Materials and Methods

The methodological approach for developing this inventory was to examine all of the current existing regulations and standards relating to food, feed, and non-food biobased applications to determine their relevance for the InnoProtein project. This was done by conducting keyword searches using the following databases: the national food safety authorities of the partner countries, EUR-Lex, FAOLEX, Codex Alimentarius and national, European, and international standards organisations including CEN and ISO. From this the relevant regulations and standards relating to the project could be grouped into the following categories, general regulations that cover the broader aspects of the project and specific regulations relating to food, feed, and non-food biobased applications.

The InnoProtein regulation inventory document includes a summary of each relevant regulation and standard. An online link has also been included providing the partners with direct access to each document. The work packages, specific tasks, and partners responsible for these tasks have been clearly identified for each regulation.

4.3. Results

The following inventory provides the required regulations, as well as the recommended standards and guidelines for the InnoProtein project.

4.3.1. Regulations

4.3.1.1. General Regulations

4.3.1.1.1. General Food Law

REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Summary:
This Regulation provides the basis for the assurance of a high level of protection of human health and consumers' interest in relation to food, considering the diversity in the supply of food including traditional products, whilst ensuring the effective functioning of the internal market. It establishes common principles and responsibilities, the means to provide a strong science base, efficient organisational arrangements, and procedures to underpin decision making in matters of food and feed safety. It also lays down the general principles that govern food and feed, particularly with regards to their safety at Community and national level. This regulation also establishes the European Food Safety Authority (EFSA) and lays down procedures for matters that impact food and feed safety, either directly or indirectly (European Parliament and Council, 2002).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002R0178-20220701

Table 9. General food law

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1., 6.2. & 6.4.	TU DUBLIN, SD, A4F, BIOTREND, NST, CHITIN & TEC
9	9.1.1., 9.1.2., 9.1.3., & 9.1.4.	TEC, VRI, NST, SD, BZN, EUFIC and TU DUBLIN

4.3.1.1.2. Microbial Criteria

COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

Summary:
This regulation sets down microbiological criteria for certain microorganisms. It implements rules that must be complied with by food business operators when implementing specific measures referred to in Regulation (EC) No 852/2004. Compliance with these rules and criteria must be verified by the competent authorities. These authorities have the right to undertake further sampling and analyses with the purpose of detection and measurement of other microorganisms and their toxins and metabolites as a means of verification of suspected unsafe food, or in the context of risk analysis (EC, 2005)

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&qid=1696255724835

Table 10. Microbial criteria

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1., 3.2. & 3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1., 5.2. & 5.3.	CHITIN & TEC
6	6.1. & 6.2.	TU DUBLIN & SD
9	9.1.3.	BZN

4.3.1.1.3. Transparency and Sustainability

REGULATION (EU) 2019/1381 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC.

Summary:
This regulation sets out to ensure that there is transparency and sustainability in the EUs risk assessment of the food chain. It considers the roles of risk assessors and risk managers. This risk communication shall seek to do the following:

- Raise awareness and understanding of specific issues under consideration.
- Ensure consistency, transparency, and clarity in the formulation of risk management recommendations and decisions.
- Provide a sound basis for understanding risk management decisions.
- Improve effectiveness and efficiency of risk management.
- Foster public understanding of risk analysis
- Ensure that the consumer, food and feed businesses and academic community are involved appropriately.
- Ensure appropriate and transparent exchange of information.
- Provision of information to consumers about strategies for risk prevention
- Fight against dissemination of false information and their sources

(European Parliament and Council, 2019).

Link:https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1381&qid=1696278388740

Table 11. Transparency and Sustainability

Work Package	Task	Relevant Partners
1	1.1., 1.2., 1.3. & 1.4.	EUFIC, BZN, ZERO-E & TU DUBLIN
7	7.1., 7.2., 7.3. & 7.4.	ZERO-E, BIOTREND, A4F, NST, TEC, SD & AIMPLAS
10	10.1, 10.2, 10.3, 10.4. & 10.5.	EUFIC, BIOTREND & A4F

4.3.1.2. Food Regulations

4.3.1.2.1. Addition of Vitamins, Minerals and Other Substances

REGULATION (EC) No 1925/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to food

Summary:
This regulation sets out the following:

- EU rules relating to the addition of vitamins, minerals and other substances to food.
- Harmonises the rules of EU member states, allowing for easier trade within the EU while ensuring consumer protection.
- Lists the vitamins and minerals that may be added to food.
- Lays down rules for additional labelling for consumers to be better informed.

(European Parliament and Council, 2006).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1925

Table 12.Addition of Vitamins, Minerals and Other Substances

Work Package	Task	Relevant Partners
9	9.1.3	BZN & EUFIC

4.3.1.2.2. Extraction Solvents

DIRECTIVE 2009/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients.

Summary:
This directive applies to the use or intended use of extraction solvents in the production of foodstuffs and food ingredients. It does not apply to solvents used to produce food additives, vitamins, and other nutritional additives, unless the food additives, vitamins or nutritional additives have been listed. This directive also ensures that the use of food additives, vitamins or nutritional additives does not result in foodstuffs containing levels of extraction solvent residue that is dangerous to human health (European Parliament and Council, 2009)

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009L0032-20230216

Table 13.Extraction Solvents

Work Package	Task	Relevant Partners
2	2.2.	TEC, BIOTREND & A4F
3	3.2.	TEC & BIOTREND
4	4.2.	TEC & NST
5	5.2.	TEC & CHITIN

4.3.1.2.3. Hygiene of Foodstuffs

REGULATION (EC) No 852/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on the hygiene of foodstuffs

Summary:
This regulation sets out the general rules for food business operators on the hygiene of foodstuffs. It pays consideration to the following principles:

- Food safety is the primary responsibility of the food business operator.
- Necessary to ensure food safety occurs throughout the food chain.
- The cold chain must be maintained for foods that cannot be stored at ambient temperatures.

- Responsibility of food business operator to enforce the implementation of HACCP procedures and the application of good hygiene practice.
- Necessary to establish microbiological criteria and temperature requirements based on scientific risk assessment.
- Necessary to ensure that imported foods are of the same or equivalent hygiene standard, at least, as the food produced in the Community.

This regulation applies to all stages of production, processing, and distribution of food (European Parliament and Council, 2004).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0852&qid=1696249284490

Table 14.Hygiene of Foodstuffs

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1., 6.2. & 6.4.	TU DUBLIN, SD, A4F, BIOTREND, NST, CHITIN & TEC
9	9.1.1., 9.1.2., 9.1.3., & 9.1.4.	TEC, VRI, NST, SD, BZN, EUFIC and TU DUBLIN

4.3.1.2.4. Labelling Laws

DIRECTIVE 2000/13/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation, and advertising of foodstuffs.

Summary:

This directive relates to the labelling of foodstuffs that are intended to be provided to the consumer and certain aspects relating to the presentation and advertising thereof. It shall also apply to foodstuffs intended to be supplied to restaurants, hospitals, canteens, and other similar mass caterers (European Parliament and Council, 2000).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0013

Table 15. Labelling Laws

Work Package	Task	Relevant Partners
9	9.1.3	BZN & EUFIC

4.3.1.2.5. Maximum Levels for Certain Contaminants

COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006

Summary:
This regulation lays down the maximum limits for certain contaminants in foodstuffs, ensuring that the health of all EU citizens is protected. Foodstuffs listed in the Annex of this regulation shall not be placed on the market if they are found to contain a listed contaminant, exceeding the maximum level. The maximum levels shall apply to food as placed on the market and to the edible part of the foodstuffs concerned (EC, 2023).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R0915

Table 16. Maximum Levels for Certain Contaminants

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1. & 6.2.	TU DUBLIN & SD
9	9.1.3. & 9.2.1.	BZN, NST, TEC & SD

4.3.1.2.6. Maximum Pesticide Residue Levels

Regulation (EC) No 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

Summary:

The purpose of this regulation is to ensure a high level of consumer protection as well as harmonising Community provisions regarding the maximum levels of pesticide residue in or on food and feed. It applies to products of plant or animal origin that are intended to be used as fresh, processed, or composite food or feed (European Parliament and Council, 2005).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0396

Table 17. Maximum Pesticide Residue Levels

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN

4.3.1.2.7. Mycotoxin Sampling and Analysis

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of levels of mycotoxins in foodstuffs.

Summary:

This regulation sets out the sampling and analysis methods of mycotoxin levels in foodstuffs, the maximum limits of which have been laid down in Regulation (EC) No 466/2001 (EC, 2006).

Link: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006R0401

Table 18. Mycotoxin Sampling and Analysis

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN

4.3.1.2.8. Novel Foods

REGULATION (EU) 2015/2283 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001

Summary:
This regulation sets out the rules and requirements for placing novel foods on the EU market. Its aim is to ensure the effective functioning of the internal market while providing a high level of protection of human health and consumer interests (European Parliament and Council, 2015).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283&qid=1699207049652

Table 19. Novel Foods

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN
9	9.1.1., 9.1.2., 9.1.3., & 9.1.4.	TEC, VRI, NST, SD, BZN, EUFIC & TU DUBLIN

4.3.1.2.9. Nutrition and Health Claims

REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on nutrition and health claims made on foods.

Summary:
The purpose of this regulation is to harmonise the provisions put in place by law, regulation or administrative action in Member States relating to nutrition and health claims, ensuring the effective functioning of the internal market while providing a high level of consumer protection. It applies to nutrition and health claims made in commercial communications, including the labelling, presentation, or advertising of foods to be delivered as such to the final consumer (European Parliament and Council, 2006).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1924&qid=1696328626981

Table 20. Nutrition and Health Claims

Work Package	Task	Relevant Partners
9	9.1.1 to 9.1.4.	BZN, TU DUBLIN, TEC, EUFIC, SD, VRI & NST

4.3.1.2.10. Nutrition Labelling for Foodstuffs

Council Directive (90/496/EEC) of 24 September 1990, on nutrition labelling for foodstuffs

Summary:
This directive is concerned with the nutritional labelling of foodstuffs to be made available to the consumer. It also applies to foodstuffs that are intended to supply restaurants, hospitals, canteens, and other mass caterers. This directive does not apply to natural mineral waters or other waters that are intended for human consumption, as well as food supplements (EEC, 1990).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31990L0496

Table 21. Nutrition Labelling for Foodstuffs

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.1.2.11. Permitted Health Claims on Food

COMMISSION REGULATION (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

Summary:
This regulation provides a list of claims that may be permitted on foods, referred to in Regulation (EC) No 1924/2006. The list provided relates to a number of nutrients and substances, as well as certain foods and food categories. It sets out the conditions of use for each claim and provides restrictions of use or additional warning statements that may be required (EC, 2012).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0432

Table 22. Permitted Health Claims on Food

Work Package	Task	Relevant Partners
9	9.1.1., 9.1.2. & 9.1.3.	TEC, VRI, NST, SD, BZN & EUFIC

4.3.1.2.12. Providing Food Information to Consumers

REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004

Summary:

The purpose of this regulation is to provide a basis for the assurance of a high level of consumer protection relating to food information, considering the differences in the perception of consumers and their information needs, as well as ensuring the effective functioning of the internal market. This regulation establishes the general principles, requirements and responsibilities governing food information, particularly food labelling. It also lays down the means to guarantee the right of consumers to information and procedures for the provision of food information, considering the need for the provision of sufficient flexibility to respond to future developments and new information requirements (European Parliament and Council, 2011).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&qid=1696262456942

Table 23. Providing Food Information to Consumers

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.1.2.13. Rules for Applying for Authorisation of Health Claims

COMMISSION REGULATION (EC) No 353/2008 of 18 April 2008 establishing implementing rules for applications for authorisation of health claims as provided for in Article 15 of Regulation (EC) No 1924/2006 of the European Parliament and of the Council

Summary:

This regulation applies to health claims that relate to the consumption of a food category, a food, or its constituents including a nutrient or other substance, or a combination of both, hereafter referred to as food. The purpose of this regulation is to ensure that health claim authorisation applications can adequately and sufficiently demonstrate the basis for the health claim and substantiate the claim with accepted scientific evidence, considering the scientific data available (EC, 2008).

Link: eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0353

Table 24.Rules for Applying for Authorisation of Health Claims

Work Package	Task	Relevant Partners
6	6.1.	TU DUBLIN
9	9.1.1., 9.1.2. & 9.1.3.	TEC, VRI, NST, SD, BZN & EUFIC

4.3.1.2.14. Trace Elements and Processing Contaminants

COMMISSION REGULATION (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the control of the levels of trace elements and processing contaminants in foodstuffs.

Summary:
The purpose of this regulation is to set out methods of sampling and analysis for the control of the levels of trace elements and processing contaminants in foodstuffs (EC, 2007).

Link:https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02007R0333-20230101&qid=1696277607589

Table 25. Trace Elements and Processing Contaminants

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN

4.3.1.3. Feed Regulations

4.3.1.3.1. Additives for Use in Animal Nutrition

REGULATION (EC) No 1831/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2003 on additives for use in animal nutrition

Summary:
This regulation aims to establish standardised authorisation procedures for the use of feed additives in animal nutrition, as well as the placing of these additives on the market. It also puts in place rules regarding the supervision and labelling of feed additives and premixtures, providing the assurance of a high level of protection of human health, animal health and welfare, and the environment while ensuring the internal market is functioning effectively (European Parliament and Council, 2003).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1831&qid=1699208843980

Table 26. Additives for Use in Animal Nutrition

Work Package	Task	Relevant Partners
9	9.2.1 & 9.2.2	NST, TEC, SD & VRI

4.3.1.3.2. Assessment and Authorisation of Feed Additives

COMMISSION REGULATION (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives.

Summary:
This regulation establishes implementing rules concerned with the procedures for authorisation of feed additives as set out in Regulation (EC) No 1831/2003. These implementing rules apply to the preparation and presentation of applications, as well as the assessment and authorisation of feed additives. These rules allow for the requirements of the accompanying dossier to be satisfied. All measures provided in this regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health (EC, 2008).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0429

Table 27. Assessment and Authorisation of Feed Additives

Work Package	Task	Relevant Partners
9	9.2.1 & 9.2.2	NST, TEC, SD & VRI

4.3.1.3.3. Fishery and Aquaculture Products

Regulation (EU) No 1379/2013 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000

Summary:
The purpose of this regulation is to establish a common market in the EU for fishery and aquaculture products. It sets out to ensure that there is an orderly and sustainable development of fishery and aquaculture sectors. The regulation provides a framework for the functioning of the market and includes rules relating to the production, processing, marketing, and trade of these products (European Parliament and Council, 2013).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R1379

Table 28. Fishery and Aquaculture Products

Work Package	Task	Relevant Partners
9	9.2.1 & 9.2.2	NST, TEC, SD & VRI

4.3.1.3.4. Protection of Pigs

COUNCIL DIRECTIVE 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs.

Summary:
This directive sets out the minimum standards for the protection of pigs kept for rearing and fattening in order to ensure rational development of production. It puts in place measures the must be implemented that fulfil pigs' basic needs and that prevent aggression among the animals (EC, 2008).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0120&qid=1696256728860

Table 29. Protection of Pigs

Work Package	Task	Relevant Partners
9	9.2.2	VRI

4.3.1.3.5. Placing on Market and Use of Feed

REGULATION (EC) No 767/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC.

Summary:
This regulation sets out rules regarding the placing on the market and use of feed for both food-producing and non-food producing animals within the Community. This includes the requirements relating to labelling, packaging, and presentation. The regulation applies, without prejudice, to other Community provisions applicable in the field of animal nutrition (European Parliament and Council, 2009)

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R0767&qid=1696261314373

Table 30. Placing on Market and Use of Feed

Work Package	Task	Relevant Partners
9	9.2.1., 9.2.2. & 9.2.3.	VRI, SD, TEC, NST & TU DUBLIN

4.3.1.3.6. Protection of Animals at Slaughter

COUNCIL REGULATION (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing.

Summary:
This regulation sets out rules for killing that have been bred or kept for the purpose of food production, wool, skin, and other products or for the purpose of depopulation. With regards to fish only the requirements set down in Article 3(1) of the regulation shall apply (EC, 2009).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1099

Table 31. Protection of Animals at Slaughter

Work Package	Task	Relevant Partners
9	9.2.2	VRI

4.3.1.3.7. Protection of Animals for Scientific Purposes

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes.

Summary:
This directive establishes measures for the protection of animals used for scientific or educational purposes, setting down the following rules:

- Replacement and reduction of the use of animals on procedures and the refinement of the breeding, accommodation, care and use of animals in procedures.
- Origin, breeding, marking, care and accommodation and killing of animals.
- Operations of breeders, suppliers and users.
- Evaluation and authorisation of projects involving the use of animals in procedures.

The directive is also applied where animals are used or intended to be used in procedures, or bred specifically so that their organs or tissues may be used for scientific purposes (European Parliament and Council, 2010).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&qid=1696261968178

Table 32. Protection of Animals for Scientific Purposes

Work Package	Task	Relevant Partners
9	9.2.1. & 9.2.2	VRI, NST, TEC & SD

4.3.1.3.8. Provisions on Processed Animal Protein

COUNCIL REGULATION (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards to the provisions on processed animal protein.

Summary:
This regulation sets out the requirements for the use of farmed insects as a source of protein for animal consumption (EC, 2017).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0893

Table 33. Provisions on Processed Animal Protein

Work Package	Task	Relevant Partners
5	5.1. to 5.3.	CHITIN & TEC
6	6.1. & 6.2.	TU DUBLIN, SD, PROMIC, A4F, BIOTREND, NST & TEC
9	9.2.1. & 9.2.2.	NST, TEC, SD & VRI

4.3.1.3.9. Requirements For Feed Hygiene

REGULATION (EC) No 183/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 January 2005 laying down requirements for feed hygiene.

Summary:
This regulation lays down the following:

- The general rules on feed hygiene
- The conditions and arrangements ensuring traceability of feed.
- The conditions and arrangements for the registration and approval of establishments

It applies to the activities of feed business operators at all stages of the feed processing chain, including the placing of feed on the market. It also applies to the feeding of food producing animals, as well as imports and exports of feed from and to third countries (European Parliament and Council, 2005).

Link:https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0183&qid=1696277167783

Table 34. Requirements For Feed Hygiene

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
9	9.2.1. to 9.2.3.	VRI, SD, TEC, NST & TU DUBLIN

4.3.1.3.10. Undesirable Substances in Animal Feed

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed

Summary:

This directive applies to all products that are intended for animal feed, this will include feed raw materials, additives, and complimentary feedstuffs. The directive sets down a list of undesirable substances and also specifies the maximum limit values for these substances in the animal feed (European Parliament and Council, 2002).

Link:https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0032-20191128&qid=1696278715653

Table 35. Undesirable Substances in Animal Feed

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN
9	9.2.1.	NST, TEC & SD

4.3.1.4. Non-Food Based Regulations

4.3.1.4.1. Financial Rules

REGULATION (EU, Euratom) 2018/1046 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 July 2018 on the financial rules applicable to the general budget of the Union, amending Regulations (EU) No 1296/2013, (EU) No 1301/2013, (EU) No 1303/2013, (EU) No 1304/2013, (EU) No 1309/2013, (EU) No 1316/2013, (EU) No 223/2014, (EU) No 283/2014, and Decision No 541/2014/EU and repealing Regulation (EU, Euratom) No 966/2012.

Summary:

The aim of this regulation is to replace previous budgetary procedures by simplify the rules for creating and implementing the EU budget within a single document. This regulation allows applicant to apply for EU funding more efficiently. It aims to avoid duplication and numerous controls by implementing a single audit approach. This regulation also strengthens the current measures that in place to protect against fraud (European Parliament and Council, 2018).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R1046

Table 36. Financial Rules

Work Package	Task	Relevant Partners
11	11.4.	TEC

4.3.1.4.1. General Data Protection Regulation (GDPR)

REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data and repealing Directive 95/46/EC (General Data Protection Regulation).

Summary:

The purpose of the GDPR is to provide protection to individuals whose data is being processed within the public and private sectors. It provides these individuals with increased control of their personal data. The GDPR sets up a system of independent supervisory authorities who are responsible for monitoring and enforcing compliance with GDPR (European Parliament and Council, 2016).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0679

Table 37. General Data Protection Regulation (GDPR)

Work Package	Task	Relevant Partners
7	7.3.	ZERO-E
9	9.1.3.	BZN & EUFIC
10	10.1. to 10.5.	EUFIC, BIOTREND, A4F & TU DUBLIN
11	11.1. to 11.4.	TEC

4.3.1.4.2. GMP For Food Contact Materials

COMMISSION REGULATION (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food.

Summary:
This regulation sets out the rules of good manufacturing practices (GMP) for the groups of materials and articles that are intended to come into contact with food, as well as combinations of materials and articles and recycled materials and articles. This regulation applies to all sectors and to all stages manufacturing, processing and distribution of materials and articles, with the exclusion of the production of starting substances (EC, 2023).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R2023&qid=1696248744459

Table 38. GMP For Food Contact Materials

Work Package	Task	Relevant Partners
6	6.3.3.	AIMPLAS
9	9.3.1.	AIMPLAS

4.3.1.4.3. Market Availability of EU Fertilising Products

REGULATION (EU) 2019/1009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003

Summary:
This regulation applies to fertilising products in the EU, including plant bio stimulants (European Parliament and Council, 2019).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1009&qid=1697208654641

Table 39. Market Availability of EU Fertilising Products

Work Package	Task	Relevant Partners
6	6.3.4.	SCL
9	9.3.2.	SCL

4.3.1.4.4. Materials and Articles Intended for Food Contact

REGULATION (EC) No 1935/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC.

Summary:
This regulation aims to ensure the effective functioning of the internal market relating to the placing on the market in the Community of materials and articles that are intended to come in contact with food, either directly or indirectly, providing the basis for securing a high level of protection of human health and consumer interests. This regulation applies to materials and articles, including active and intelligent food contact materials, that in their finished state are:

- Intended to be brought into contact with food.
- Already in contact with food and are intended or that purpose.
- Reasonably expected to be brought into contact with food or transfer constituents to food under normal or foreseeable conditions.

(European Parliament and Council, 2004).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R1935&qid=1696249736257

Table 40. Materials and Articles Intended for Food Contact

Work Package	Task	Relevant Partners
6	6.3.3.	AIMPLAS
9	9.3.1.	AIMPLAS

4.3.1.4.5. Single Use Plastics

DIRECTIVE (EU) 2019/904 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 June 2019 on the reduction of the impact of certain plastic products on the environment

Summary:
The purpose of this directive is to prevent and reduce the impact of plastics on the environment and human health by promoting the transition to a circular economy through innovation and sustainability. This directive applies to single-use plastics, products made from oxo-degradable plastics and to fishing gear containing plastic (European Parliament and Council, 2019).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019L0904

Table 41. Single Use Plastics

Work Package	Task	Relevant Partners
6	6.3.3.	AIMPLAS
9	9.3.1.	AIMPLAS

4.3.1.4.6. Waste Management

DIRECTIVE 2008/98/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 November 2008, on waste and repealing certain Directives.

Summary:
This directive lays down measures to protect the environment and human health by preventing or reducing the adverse impacts of the generation and management of waste and by reducing overall impacts of resource use and improving the efficiency of such use (European Parliament and Council, 2008).

Link:https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0098&from=EN

Table 42. Waste Management

Work Package	Task	Relevant Partners
2	2.2.	TEC, BIOTREND & A4F
3	3.2.	TEC & BIOTREND
4	4.2.	TEC & NST
5	5.2.	TEC & CHITIN
6	6.2. & 6.3.	SD, PROMIC, A4F, BIOTREND, NST, AIMPLAS, CHITIN, TEC & SCL

4.3.2. Standards

4.3.2.1. Food

4.3.2.1.1. Contaminants and Toxins

CXS 193-1995 General Standard for Contaminants and Toxins in Food and Feed

Summary:
This standard provides the main principles recommended by Codex Alimentarius regarding contaminants and toxins in food and feed. It also provides a list of the maximum levels and sampling plans for contaminants and natural toxicants (Codex Alimentarius, 2019).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf

Table 43. Contaminants and Toxins

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1.	TU DUBLIN
9	9.2.1.	NST, TEC & SD

4.3.2.1.2. Labelling of Food Additives

CXS 107-1981 General Standard for the Labelling of Food Additives When Sold as Such

Summary:
This standard is applied to the labelling of food additives sold as additives, either by retail or other means. This can include sales to caterers and food manufacturers for the purpose of their business. Furthermore, this standard can also be applied to processing aids, or any reference to food additives including food processing aids (Codex Alimentarius, 2016).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B107-1981%252FCXS_107e.pdf

Table 44. Labelling of Food Additives

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.2.1.3. Labelling of Prepackaged Foods

CXS 1-1985 General Standard for the Labelling of Prepackaged Foods

Summary:
This standard is applied to the labelling of all prepackaged foods intended to direct use by the consumer, or for catering purposes (Codex Alimentarius, 2018).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B1-1985%252FCXS_001e.pdf

Table 45. Labelling of Prepackaged Foods

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.2.1.4. Methods of Analysis

CXS 228-2001 General Methods of Analysis for Contaminants

Summary:

This document provides standard methods of analysis for heavy metal contaminants in foods (Codex Alimentarius, 2001).

Link: : https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B228-2001%252FCXS_228e.pdf

Table 46. Methods of Analysis

Work Package	Task	Relevant Partners
6	6.1.	TU DUBLIN

4.3.2.2. Non-Food Based Applications

4.3.2.2.1. Life Cycle Assessment

ISO 14040:2006 Environmental management: Life cycle assessment – Principles and framework

Summary:
The purpose of this standard is to provide the principles and framework for life cycle assessment (LCA) (ISO, 2006).

Link: https://www.iso.org/standard/37456.html

Table 47. Life Cycle Assessment

Work Package	Task	Relevant Partners
7	7.1. to 7.4.	ZERO-E, BIOTREND, A4F, NST, TEC, SD, PROMIC & AIMPLAS

4.3.2.2.2. Plant Biostimulants

CEN/TS 17700-1:2022 Plant Biostimulants – Claims – Part 1: General Principles

Summary:
This standard sets put the general principles for the justification of the claims made on plant biostimulant products. These principles outline the requirements that are necessary when assessing the efficacy of trials conducted on plant biostimulants when validating such claims (CEN, 2022).

Link: https://standards.cencenelec.eu/dyn/www/f?p=205:110:0::::FSP_ORG_ID,FSP_LANG_ID:2279055,25&cs=11A30216CC050D84360A3A54EDC84A923

Table 48. Plant Biostimulants

Work Package	Task	Relevant Partners
6	6.3.4.	SCL
9	9.3.2.	SCL

4.3.3. Guidelines

4.3.3.1. Food

4.3.3.1.1. Addition of Essential Nutrients to Food

CAC/GL 9-1987 General Principles for the Addition of Essential Nutrients to Foods

Summary:
The principles set out in this document aim to be applied to all foods to which essential nutrients are added, excluding vitamin and mineral supplements. These principles are applicable to the mandatory and voluntary addition of essential nutrients to foods (Codex Alimentarius, 2015)
.Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B9-1987%252FCXG_009e_2015.pdf

Table 49. Addition of Essential Nutrients to Food

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.3.1.2. Food Hygiene

CXC 1-1969 General Principles of Food Hygiene

Summary:
The purpose of this document is to provide a general principle framework for the safe production and suitability of food. It also outlines the necessary hygiene and food safety controls that need to be implemented throughout the food chain (Codex Alimentarius, 2022).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B1-1969%252FCXC_001e.pdf

Table 50. Food Hygiene

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1.,3.2. &3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1.,5.2. & 5.3.	CHITIN & TEC
6	6.1., 6.2. & 6.4.	TU DUBLIN, SD, A4F, BIOTREND, NST, CHITIN & TEC
9	9.1.1., 9.1.2., 9.1.3., & 9.1.4.	TEC, VRI, NST, SD, BZN, EUFIC and TU DUBLIN

4.3.3.1.3. Microbiological Criteria Guidelines

CXG 21-1997 Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods

Summary:
The purpose of this document is to provide national authorities and FBOs with a framework to establish and apply microbiological criteria to food safety and other aspects of food hygiene (Codex Alimentarius, 1997).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B21-1997%252FCXG_021e.pdf

Table 51. Microbiological Criteria Guidelines

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1., 3.2. & 3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1., 5.2. & 5.3.	CHITIN & TEC
6	6.1. & 6.2.	TU DUBLIN & SD
9	9.1.3.	BZN

4.3.3.1.4. Nutrition and Health Claim Guidelines

CAC/GL 23-1997 Guidelines for Use of Nutrition and Health Claims

Summary:
The guidelines set out in this document are related to the use of nutrition and health claims in food labelling and in advertising, where required. These guidelines apply to all foods for which nutrition and health claims have been made (Codex Alimentarius, 1997).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B23-1997%252FCXG_023e.pdf

Table 52. Nutrition and Health Claim Guidelines

Work Package	Task	Relevant Partners
9	9.1.1 to 9.1.4.	BZN, TU DUBLIN, TEC, EUFIC, SD, VRI & NST

4.3.3.1.5. Nutrition Labelling

CXG 2-1985 Guidelines on Nutrition Labelling

Summary:
The purpose of these guidelines is to make recommendations relating to the procedures for the nutrition labelling of foods and apply to the nutritional labelling of all foods (Codex Alimentarius, 2021).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B2-1985%252FCXG_002e.pdf

Table 53. Nutrition Labelling

Work Package	Task	Relevant Partners
9	9.1.3.	BZN & EUFIC

4.3.3.2. Feed

4.3.3.2.1. Fish and Fishery Products

CXC 52-2003 Code of Practice for Fish and Fishery Products

Summary:
This Code applies to the growing, harvesting, handling, production, processing, storage, transportation and retail of fish, shellfish and aquatic invertebrates and products thereof from marine and freshwater sources that are intended for human consumption (Codex Alimentarius, 2003)

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B52-2003%252FCXC_052e.pdf

Table 54. Fish and Fishery Products

Work Package	Task	Relevant Partners
9	9.2.1. & 9.2.2.	VRI, SD, TEC & NST

4.3.3.2.2. Good Animal Feeding Code of Practice

CAC/RCP 54-2004 Code of Practice on Good Animal Feeding

Summary:
The aim of this document is to establish a feed safety system for food producing animals, covering the entire food chain. This code considers the relevant aspects of animal health and the environment as a means of minimising the risk to consumer health. This code is applied in addition to the principles of food hygiene, taking in to account the special aspects of animal feeding (Codex Alimentarius, 2004).

Link: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/de/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B54-2004%252FCXP_054e.pdf

Table 55. Good Animal Feeding Code of Practice

Work Package	Task	Relevant Partners
2	2.1.,2.2. & 2.3.	A4F, BIOTREND & TEC
3	3.1., 3.2. & 3.3.	BIOTREND & TEC
4	4.1., 4.2. & 4.3.	NST & TEC
5	5.1., 5.2. & 5.3.	CHITIN & TEC
9	9.2.1. & 9.2.2.	VRI, SD, TEC & NST

4.3.3.3. Non-Food Based Applications

4.3.3.3.1. EU Policy Framework on Biobased, Biodegradable and Compostable Plastics

Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions on the EU policy framework on biobased, biodegradable, and compostable plastics.

Summary:
The aim of this EU policy framework is to help the sustainable plastics economy by:

- Improving understanding of these materials and how they can benefit the environment

- Providing guidance to consumers, public authorities and businesses about policy decisions
- Promoting the standardisation of the prodction and use of these plastics across the EU

(EC, 2022)

Link: https://environment.ec.europa.eu/system/files/2022-12/COM_2022_682_1_EN_ACT_part1_v4.pdf

Table 56. EU Policy Framework on Biobased, Biodegradable and Compostable Plastics

Work Package	Task	Relevant Partners
9	9.2.1. & 9.2.2.	VRI, SD, TEC & NST

4.3.3.3.2. Life Cycle Environmental Performance

Commission Recommendation (EU) 2021/2279 of 15 December 2021 on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations.

Summary:
The purpose of this recommendation is to promote the use of Environmental Footprint methods in relevant policies and schemes related to measurement and communication of the life cycle environmental performance of products, services, and organisations. This recommendation is aimed at EU member states, as well as public and private organisations (EC, 2021).

Link: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021H2279

Table 57. Life Cycle Environmental Performance

Work Package	Task	Relevant Partners
7	7.1. to 7.4.	ZERO-E, BIOTREND, A4F, NST, TEC, SD, PROMIC & AIMPLAS

4.4. Conclusion

This inventory developed by TU Dublin for the InnoProtein project is quite extensive. It includes a wide range of regulations and standards relevant to the project, some relating to the broader aspects of the project while others are more specific to certain tasks and project partners. The overall aim of this regulatory inventory is to ensure that all project partners are compliant with the requirements of EU legislation and relevant standards while conducting their work throughout the project. Total compliance with all of these regulations and standards will help in achieving EU and international market approval for the protein-based products that are planned to be developed during the InnoProtein project. Moreover, compliance with these regulations and standards will, most importantly, ensure a high degree of safety for the proposed end users, namely humans and animals.

As the InnoProtein project seeks to investigate the utilization of unconventional protein sources, specifically single cell proteins (SCPs) and insects, for food, feed and non-food biobased applications meticulous scientific based safety assessments must be conducted. These must be done in accordance with EU legislation to ensure end user protection and limit the spread of misinformation (de Boer, 2019). One of the key pieces of legislation relating to the project is Regulation (EC) No 178/2002, General Food Law, which covers the broader details of European food law. Under this regulation the general principles and requirements of food law have been set out for the governing of food and feed safety at both national and EU level (European Parliament and Council, 2002). This provides the intended end users with certain assurances that they will receive a high degree of protection with respect to their health, safety, and well-being. However, while the primary objective of EU food legislation is to protect consumer health and interests the legislation in place does not allow any specific rights to the consumer (van der Meulen, 2013). As such the onus falls on the food and feed business operators, at all stages of production, to ensure a high degree of safety throughout the food and feed chains.

Alongside the EU regulations this inventory also provides a considerable amount of information on ISO, CEN, and Codex Alimentarius standards and guidelines relating to food, feed, and non-food biobased applications. In terms of global food safety standards Codex Alimentarius is held in high regard, with the organisations primary focus being public health (Trienekens and Zuurbier, 2008). It is important to note that while the standards provided in this inventory are voluntary, and not tied to the legislation, they are viewed as the benchmark for good manufacturing practices (GMP), particularly within the food and feed industries. By implementing these standards in the production of InnoProtein food and feed products the partners are providing certain assurances to the end users that these protein products are safe to consume and are of a suitable quality for their intended applications.

As such, to ensure the safety of InnoProtein proteins produced for incorporation in food and feed chains a series of safety assessments must be conducted, including analysis for the presence of contaminants, such as heavy metals and pesticide residues, toxins, allergenic potential, and microbiological contamination This inventory contains a number of relevant regulations, standards and guidelines that will aid the partners when carrying out such activities.

Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food and Codex standard CXS 193-1995 both set the maximum levels of heavy metals, including lead (Pb), cadmium (Cd) and mercury (Hg). While these metals occur naturally in the environment there is a risk of heavy metal residue contamination of the food and feed chain at any stage of production. The regulation and standards provide the requirements for the maximum acceptable limit of these contaminants allowed in food the risk to human health becomes too great. Long term exposure or consumption of large quantities of heavy metals can severely impact human and animal health. Lead poisoning, for example, is a major public health concern, the effects of which can cause damage to the nervous system and organs, including the kidneys and intestines (Scutarașu and Trincă, 2023).

Regulation (EC) No 396/2005 sets down the maximum pesticide residues that are acceptable in food and animal feed. Pesticides are a major hazard for food and animal feed, as such the EU has in place strict policies in relation to the use of pesticides and their maximum residue levels (MRLs) (Kuchheuser and Birringer, 2022). There is growing concern surrounding the adverse effects of pesticides in both human and animal health, as well as the environmental impact. These pesticide MRLs are considered a perquisite for placing food and animal feed products on the EU market, ensure a high degree of end user safety.

Microbiological contamination is one of the greatest risks to food and feed and safety as microorganisms can grow and survive in a multitude of different environments. Therefore, continuous assessment of microbial content in food and feed is essential in order to protect the interests of the end users. Commission Regulation (EC) No 2073/2005 and Codex guideline CAC/GL 21-1997 both set the microbiological criteria for foodstuffs. While microbial testing does not guarantee product safety the criteria that have been set out in both of these documents provide producers with a reference point that they may use to monitor the safety of their products. Food and feed products should not contain pathogenic bacteria, including Salmonella, *Staphylococcus aureus* and *Escherichia coli,* the presence of which in these products would a cause for concern.

There are currently fourteen food ingredients listed as allergens in the EU, they are: Celery, cereals containing gluten, crustaceans, eggs, fish, lupin, milk, molluscs, mustard, peanuts, sesame, soybean, sulphur dioxide and sulphites. The presence of these allergens in foods can negatively impact the health of the certain consumers, whereby contact with an allergen can cause mild to severe symptoms, including anaphylaxis which may be fatal in some instances. Therefore, it is essential that all known allergens in a food product be presented clearly to the consumer. Directive 2000/13/EC relates to labelling laws, while Regulation (EU) No 1169/2011 relates to the provision of food information to the consumer. The purpose of these pieces of legislation is to provide assurances to the consumer that the information provided to them regarding their food is correct and that the product is safe for them to consume. The unconventional nature of these protein sources means that a majority of the SCP and insect species selected for the InnoProtein project are considered novel. As such the allergenicity of these proteins may not be well understood and further studies on these proteins will be conducted to identify any potential risks for the consumers.

The InnoProtein project has adopted a circular, zero waste approach to protein production, and as such proteins outside the spec of food and feed quality will be utilised for the production of bioplastics and biostimulants. While there does not appear to be any regulations or standards relating specifically to bioplastics as of yet, there is however an EU policy framework document which addresses the production and application of biobased, biodegradable, and compostable plastics within the EU. There are a couple of regulations provided within this inventory that will also help guide the partners when considering the production of the bioplastic products. The first is Directive (EU) 2019/904 on single use plastics, which promotes the use of sustainable, non-toxic plastic products over single use products (European Parliament and Council, 2019). Secondly, Regulation (EC) No 1935/2004 on materials intended for food contact aims to ensure that there is a high level of safety for human health when producing or using materials that are intended to come into contact with food (European Parliament and Council, 2004).

As the InnoProtein project progresses it is expected that this inventory will change. With this in mind TU Dublin will continue to monitor the regulatory requirements necessary to the project, updating this inventory and informing all project partners of any changes to the regulations, standards, and guidelines as they happen.

Conclusion

Deliverable D1.1 has established the sustainable requirements for the unconventional proteins that are to be used as part of the InnoProtein project. The social requirements for these proteins have been established, and their importance in the development of new sustainable proteins has been highlighted. The market analysis has determined that the insect protein market currently holds the greatest market value, and this is unlikely to change in the coming years. The analysis also anticipates that the microalgae market value will grow exponentially on a global scale. The research has also highlighted that all of these proteins have widespread applications across both the food and feed sectors. However, their use in non-food biobased applications remains limited. Market research has also determined that the most prominent barrier to market entry for InnoProtein proteins is high cost of production.

Current intensive food and feed production practices have resulted in a long-term negative impact on the environment. The research has shown that there are a number of benefits to utilising SCPs and insects for the production of proteins including reduced land and water use. Microalgae, in particular, shows great potential as a sustainable alternative due to their ability to grow and thrive in a variety of environmental conditions, as well as their high protein content. However, the recovery of protein from microalgae can be a challenge. Greener extraction methods including the use of ILs and DESs are now being considered as they can allow for a more efficient and environmentally friendly alternative to protein recovery.

An inventory of regulations, standards and guidelines has been compiled, using national, EU and international databases, assessing the regulatory requirements for the InnoProtein project. The inventory is intended to act as a reference guide for those involved in the project, providing them with all of the essential information required to ensure compliance and end user protection. It is essential for the InnoProtein project that all of the sustainable requirements be established and consider during the early stages of the project as it will enable those involved to allow these proteins to meet end users needs and expectations.

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